CIBA FOUNDATION COLLOQUIA
ON ENDOCRINOLOGY

Vol. VIII. The Human Adrenal Cortex

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CIBA FOUNDATION COLLOQUIA ON ENDOCRINOLOGY

VOLUME VIII

The Human Adrenal Cortex

Editors for the Ciba Foundation

G. E. W. WOLSTENHOLME, O.B.E., M.A., M.B., B.Ch.

and

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With 227 Illustrations



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THE CIBA FOUNDATION

or the Promotion of International Co-operation in Medical and Chemical Research

41 Portland Place, London, W.1.

PREFACE

The subject of the conference of which this volume contains the proceedings was suggested jointly by Dr. Gregory Pincus and Prof. G. W. Thorn. The Ciba Foundation had already devoted 12 of its previous 25 small international conferences to the consideration of endocrinological problems, and the Director gladly agreed to provide a further opportunity for discussion of hormonal research, this time on the human adrenal cortex.

The extent of the material to be considered, and the extreme difficulty of limiting the membership in so active a field to a number at which informal discussions in a round-table manner could be obtained, led to a division of the Colloquium into two halves: the first, concerned mainly with histological and biochemical aspects and cortico-medullary relationships, being held in April under the chairmanship of Dr. Pincus; and the second, on physiological and pathological aspects and hypothalamic and pituitary relationships, taking place in June with Dr. Thorn in the chair. To both the Chairmen the Director of the Ciba Foundation is deeply indebted, not only for the original idea of the Colloquium and for presiding over it, but also for much information and advice on its organisation.

Though primarily concerned with the *human* adrenal cortex, neither the Chairmen nor the Director had any wish to exclude useful supporting information from animal experiments. The reader will also observe that considerable latitude, in reference to animal work and to more general endocrinological physiology, was permitted to the authors who were considering the influence of hypothalamus and pituitary on the adrenal cortex.

To those to whom this book serves as an introduction to the activities of the Ciba Foundation it should be explained that it is an international centre, which is established as an

educational and scientific charity under the laws of England. It owes its inception and support to its founder, Ciba Ltd. of Switzerland, but is administered independently and exclusively by its distinguished British Trustees.

The Foundation provides accommodation for scientific workers who visit London from abroad, organizes and holds international symposia, conducts (in conjunction with the Institut National d'Hygiène) a postgraduate medical exchange scheme between England and France, arranges informal meetings for discussions, awards an annual lectureship, has embarked on a scheme to encourage basic research relevant to the problems of ageing, assists international congresses and other scientific societies, is building up a library service in special fields, and generally endeavours to give aid in all matters that may promote international co-operation in scientific research.

Leading research workers from different countries and in different disciplines are invited to attend the symposia or colloquia. The size of the groups is, however, very strictly limited in order to obtain a free conversational manner of discussion—although the basic timetable of the programme is strictly observed. The smallness of the groups means the exclusion of many other workers active and interested in the subjects discussed, and therefore the proceedings of these conferences are published and made available throughout the world.

It is hoped that the papers and discussions in this book will prove not only informative and stimulating, but will also give to readers a sense of participation in an informal and friendly occasion.

CONTENTS

Part I

Chairman: GREGORY PINCUS

Part II

Chairman: GEORGE W. THORN

List of those participating in or attending Part I of the Colloquium on "The Human Adrenal Cortex", 21st–23rd April 1954

C. R. ADDINALL . . .	Merck & Co. Inc., Rahway, New Jersey
G. H. BOURNE . . .	Histology Dept., London Hospital Medical School, London
I. E. BUSH . . .	St. Mary's Hospital Medical School, London
D. B. CATER . . .	Dept. of Pathology, University of Cambridge
C. L. COPE . . .	Postgraduate Medical School of London
R. I. DORFMAN . .	Worcester Foundation for Experimental Biology, Shrewsbury, Mass.
U. S. VON EULER . .	Fysiologiska Inst., Karolinska Inst., Stockholm
S. J. FOLLEY . . .	National Inst. for Research in Dairying, Reading
R. GAUNT	Dept. of Endocrine Research, Ciba, Summit, New Jersey
C. H. GRAY . . .	Dept. of Chemical Pathology, King's College Hospital, London
MARGARETHE HOLZBAUER .	Inst. of Physiology, University of Graz, Austria, and Pharmacological Laboratory, University of Edinburgh
W. KLYNE . . .	Postgraduate Medical School of London
B. H. LANDING . .	Children's Hospital Research Foundation, Cincinnati, Ohio
J. D. LEVER . .	Dept. of Anatomy, University of Cambridge
G. F. MARRIAN . . .	Dept. of Biochemistry, Univ. of Edinburgh
C. MIGEON . . .	Dept. of Biological Chemistry, Univ. of Utah
C. J. O. R. MORRIS . .	Endocrine Unit, London Hospital, London
J. K. NORYMBERSKI . .	Centre for Investigation and Treatment of Rheumatic Diseases, Sheffield
P. OSINSKI . . .	Cliniques Universitaires St.-Pierre, Louvain
G. PINCUS . . .	Worcester Foundation for Experimental Biology, Shrewsbury, Mass.
F. T. G PRUNTY . .	Dept. of Chemical Pathology, St. Thomas's Hospital, London
G. RUMNEY . .	Hormone Research Lab., Hadassah Medical School, Jerusalem (now at McArdle Memorial Lab., *Madison, Wis.*)
SYLVIA A. SIMPSON . .	Courtauld Inst. of Biochemistry, Middlesex Hospital, London

List of those participating in or attending the Conference on Steroid Hormones and Tumour Growth, 10th to 12th July, 1950

W. T. Astbury	University of Leeds
R. W. Begg	University of Western Ontario, Canada
R. R. Bomford	London Hospital, London
E. Boyland	Chester Beatty Research Institute, London
J. H. Burchenal	Sloan-Kettering Institute, New York
H. Burrows	*late of* Chester Beatty Res. Inst., London
A. Chamorro	Institut du Radium, Paris
G. W. Corner	Carnegie Institution of Washington, U.S.A.
A. T. Cowie	National Institute for Research in Dairying, Reading
L. Dmochowski	University of Leeds
K. Dobriner	Sloan-Kettering Institute, New York
S J. Folley	National Institute for Research in Dairying, Reading
L. F. Foulds	Chester Beatty Research Institute, London
W. U. Gardner	Yale University School of Medicine
F. Gross	Ciba Limited, Basle
A. Haddow	Chester Beatty Research Institute, London
R. Hertz	National Institute of Health, Bethesda, U.S.A.
I. Hieger	Chester Beatty Research Institute, London
Lord Horder	London
E. S. Horning	Chester Beatty Research Institute, London
C. Huggins	University of Chicago
A. E. Kellie	Courtauld Institute of Biochemistry, London
R. Korteweg	Nederlandsch Kankerinstituut, Amsterdam
W. R. Lyons	University of California
O. Mühlbock	Nederlandsch Kankerinstituut, Amsterdam
B. D. Pullinger	The Glasgow Royal Cancer Hospital
C. W. Shoppee	University College of Swansea
I F. Sommerville	University of Edinburgh
C. Chester Stock	Sloan-Kettering Institute, New York
P. C. Williams	Imperial Cancer Research Fund, London

List of those participating in or attending the Conference on Steroid Hormones and Enzymes, 8th to 10th March, 1950

I. E. Bush	National Institute for Medical Research, London
P. Mary Cotes	University of Cambridge
D. H. Curnow	Animal Health and Nutrition Laboratory, Western Australia
E. C. Dodds	Courtauld Institute of Biochemistry, London
L. A. Elson	Chester Beatty Research Institute, London
W. H. Fishman	Tufts College, Boston, Mass.
S. J. Folley	National Institute for Research in Dairying, Reading
T. H. French (decd.)	National Institute for Research in Dairying, Reading
Nancy Gough	Chester Beatty Research Institute, London
A. L. Greenbaum	University College, London
Lord Horder	London
C. D. Kochakian	Oklahoma Medical Research Unit and Hospital, Oklahoma City, U.S.A.
Patricia McLean	Courtauld Institute of Biochemistry, London
W. H. H. Merivale	Guy's Hospital Medical School, London
R. K. Meyer	University of Wisconsin, U.S.A.
G. T. Mills	University of Glasgow
J. N. Smith	St. Mary's Hospital, London
A. W. Spence	St. Bartholomew's Hospital, London
I. D. E. Storey	University of Edinburgh
R. H. S. Thompson	Guy's Hospital Medical School, London
A. Tickner	Guy's Hospital Medical School, London
H. G. Williams-Ashman	Chester Beatty Research Institute, London
F. G. Young	University of Cambridge

BOOK I

STEROID HORMONES AND TUMOUR GROWTH

FOREWORD

by

Professor A. Haddow, D.Sc., Ph.D., M.D.

Of all the branches of physiology and chemistry having a direct bearing on the cancer problem, steroid endocrinology holds a key position, and is certain to figure prominently—possibly decisively—in the ultimate solution. It is not without significance, and is perhaps prophetic, that the first example of this physiological control of one type of human cancer, namely the œstrogen treatment of carcinoma of the prostate, should have sprung from this field. Even if the present situation is one of extreme complexity, with innumerable anomalies and paradoxes, the subject shows every sign of active growth, with each fresh development having an immediate impact upon one or other aspect of the tumour problem. In all these circumstances the Ciba Foundation conference on Steroid Hormones and Cancer served a valuable purpose. If recent years have seen advances, as for instance in our knowledge of the influence of steroid hormones on the appearance and behaviour of tumours of the mammary gland, pituitary and gonads, there is also need for constant revision, and it is refreshing to find, for example, that the moderately simple interpretation of an anti-androgenic mechanism in the control of cancer of the prostate, so far accepted, is itself now open to some question. The conference further provided useful discussion of the metabolic functions of the steroid hormones, of the tumour-host relationship, and of the nature and significance of alterations in the steroid excretion, leading especially to the newer view that the origin of neoplastic disease may be associated with an adrenal-gonad insufficiency of a hitherto unexpected kind. We owe a considerable debt of gratitude not only to

the Ciba Foundation itself and to those workers from the United States, Canada, the Continent and England who contributed authoritative papers, but also to Dr. G. E. W. Wolstenholme, Miss M. P. Cameron and Mr. J. M. Garratt for their work of editorship which has allowed the proceedings of the conference to be available in permanent form.

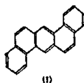
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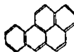
THE INDUCTION OF NORMAL AND MALIGNANT GROWTH WITH STEROIDS AND RELATED SUBSTANCES

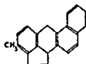
STEROIDS IN RELATION TO CANCER FROM THE CHEMICAL ASPECT

C. W. SHOPPEE

FOLLOWING the original production of malignant skin tumours in the laboratory with coal tar by Yamagiwa and Ichikawa in 1915, the work of Bloch, Passey, Leitch, Kennaway, Mayneord, Cook and Hieger led during the decade 1920-30 to the discovery of the carcinogenic hydrocarbons. 1:2:5:6-Dibenzanthracene (I), the first synthetic carcinogen, was soon succeeded in 1933 by 3:4-benzpyrene (II) and other polycyclic aromatic hydrocarbons. Because methyl-cholanthrene (III) can be regarded as a 5:6:10-trisubstituted-1:2-benzanthracene, Cook predicted that it would be carcinogenic, and Cook and Haslewood in 1934 showed it to be highly potent.


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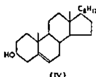

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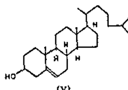
(III)

In 1932, the chemical structure of the steroids was elucidated and the formula of cholesterol (IV) settled (Wieland, Windaus, Rosenheim and King); this formula with the

addition of the stereochemical detail* shown in (V) is now completely established by the work of Wieland and Dane in 1933, Reichstein and Sorkin in 1946, and in 1947, by Shoppee and Cornforth and Robinson. Stereochemistry is immensely important in relation to biological activity.

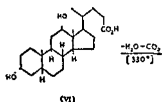


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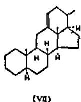


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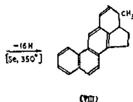
In 1933, Wieland and Dane converted deoxycholic acid (VI) via dehydronorcholene (VII) into methylcholanthrene (VIII). The high carcinogenic potency of methylcholanthrene and its close structural relationship to the steroids, which was fully appreciated by Cook even before the transformation (VI⟶VIII) was achieved, led to the suggestion associated with the names of Cook, Kennaway and Dodds that carcinogenic hydrocarbons might arise from steroids occurring naturally in the organism.



(VI)



(VII)



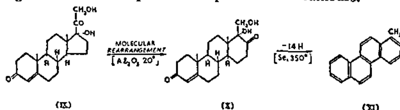
(VIII)

There is, however, no direct evidence that these reactions, although attainable in the laboratory, occur under physiological conditions. It was reported by Druckrey, Richter, and Vierthaler in 1941 that benzene extracts of *Bacillus coli* cultures grown in the presence of dehydronorcholene (VII) produced malignant tumours in rats; this activity is not due

*Full-line and broken-line bonds show the position of groups above or below the general plane of the ring system.

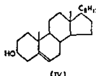
to some transformation product of dehydronorcholene because this substance, which is noncarcinogenic, is unaltered by *B. coli*, as shown by Butenandt and Dannenberg in 1950. It seems therefore that the transformation (VI⟶VIII) is not biologically significant, although (VI) may produce sarcomas in mice (Kennaway, 1940).

An alternative and smoother path for the conversion of a 17α-hydroxy-20-ketosteroid (IX), of the type produced by the adrenal cortex, *via* a D-homoandrostane derivative (X) to a carcinogenic methylchrysene (XI) was suggested by Shoppee in 1947; the stage (IX⟶X) proceeds under physiological conditions, but there is no evidence that this is also true for the complete dehydrogenation (X⟶XI), although analogies may be quoted, e.g. dehydrogenation of cyclohexanecarboxylic acid to benzoic acid in the liver (Bernhard, 1945; Dickens, 1948, 1949) and the *in vivo* dehydrogenation of (+)-œstrone to (+)-equilenin in the pregnant mare (Girard, 1932, 1933). The saturated hydrocarbon 17α-methyl-D-homoandrostane corresponding to (X) is not carcinogenic in mice by painting; the methylchrysene (XI) appears to be a weak carcinogen, for although it produced no tumours in mice by painting, by the graft technique of Horning it caused proliferation of the prostatic epithelium, accompanied by active mitosis in the glandular epithelium, with infiltration of the stroma, processes which in time might have led to tumour formation. Nevertheless, it would seem that the transformation (IX⟶XI) is not significant in regard to the development of "spontaneous" tumours.

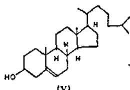


The analytical approach to the question of the production of carcinogenic hydrocarbons from naturally occurring

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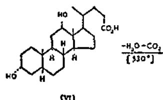


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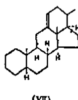
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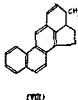
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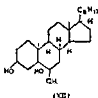
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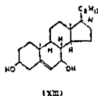
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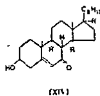
this time as to the mechanism of the connexion. The natural œstrogenic hormones induce normal growth of the uterine mucosa, but are all able to produce mammary cancer in genetically suitable male and female mice, and also pituitary, uterine, testicular, subcutaneous, leucocytic and bone tumours. The œstrogenic factor was isolated and identified by Doisy in 1936 as 3:17β-œstradiol, and the original observation of Lacassagne in 1932 for œstrone has been confirmed by Burrows in 1935, whilst experimental work leading to tumorigenesis has been reported for œstradiol (Gardner, 1936), and for equilenin and equilin (Lacassagne, 1936; Gardner, 1936). From the chemical point of view, it is not known whether œstrogens cause or mediate in cellular proliferation; their action is unspecific, inasmuch as many œstrogens of diverse and different chemical types are known, but all can produce tumours.

Reference must next be made to the striking experiments of Woolley, Fekete and Little (1940, 1945); in a strain (CE) of inbred mice, either ovariectomy of females or castration of males shortly after birth gave 100 per cent yields of adreno-cortical tumours which metastasized and were transplantable. Nothing extrinsic is administered to the mice and the tumour arises in a suitable genetic milieu by hormonal imbalance probably occasioned by adrenal hyperactivity in compensation for the absence of the gonads. Rhoads, Dobriner and their co-workers (1947) have suggested that adrenocortical hormone production and metabolism are very frequently disturbed in neoplastic disease. They have isolated from the ketonic fractions of human urines some 42 compounds, all steroids, of which at least 27 have been identified; one of these compounds, 3α-hydroxy-5-*iso*androst-9:11-ene-17-one (XV), appears to be associated with cancer. In Dr. Dobriner's words "a fair statement would seem to be that this compound is very significant but not wholly specific for cancer"; from the chemical point of view there can be no doubt that it is derived by dehydration of one of the 11β-hydroxy-steroids characteristic of the adrenal cortex, and which are subject to

steroids is by examination of lipoid-soluble material from tumour tissue, or from unaffected organs or body fluids of cancer cases; in this way evidence of the occurrence of carcinogens in human tissues has been obtained by six independent groups of workers (Schabad, 1940; des Ligneris, 1940; Hieger, 1940; Sannié *et al.*, 1941; Steiner, 1942; Menke, 1942). The tumours produced by these tissue extracts are generally of the spindle-cell type resembling those produced by carcinogenic hydrocarbons, but no compound or compounds responsible have been isolated. In an investigation of a tissue extract from Bantu livers supplied by Dr. Hieger, the writer found more than 85 per cent of cholesterol, and a little of the *n*-paraffin $C_{29}H_{60}$, but the only other compounds as yet isolated and identified were oxidation products, probably harmless, of cholesterol, namely cholestane-3β:5α:6β-triol (XII), 7-hydroxycholesterol (XIII), and 7-ketocholesterol (XIV). It is interesting to note that the triol (XII) and the ketone (XIV) are produced when cholesterol is irradiated with X-rays in an aqueous medium, whilst the diol (XIII) results from auto-oxidation of cholesterol with molecular oxygen at 20°C. in colloidal aqueous solution. The main point in the present connexion is that Dr. Hieger and the author were unable to detect in the tissue extract and after removal of the ballast material, i.e. after about a hundredfold concentration, any substance giving the type of ultra violet fluorescence spectrum characteristic of polycyclic aromatic hydrocarbons.





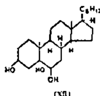


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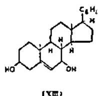
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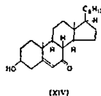
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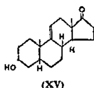
(XIV)

There is a considerable body of evidence to be presented at this conference that steroids are directly implicated in the cancer problem, although probably nothing can be said at

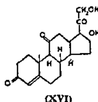
this time as to the mechanism of the connexion. The natural œstrogenic hormones induce normal growth of the uterine mucosa, but are all able to produce mammary cancer in genetically suitable male and female mice, and also pituitary, uterine, testicular, subcutaneous, leucocytic and bone tumours. The œstrogenic factor was isolated and identified by Doisy in 1936 as 3:17β-œstradiol, and the original observation of Lacassagne in 1932 for œstrone has been confirmed by Burrows in 1935, whilst experimental work leading to tumorigenesis has been reported for œstradiol (Gardner, 1936), and for equilenin and equilin (Lacassagne, 1936; Gardner, 1936). From the chemical point of view, it is not known whether œstrogens cause or mediate in cellular proliferation; their action is unspecific, inasmuch as many œstrogens of diverse and different chemical types are known, but all can produce tumours.

Reference must next be made to the striking experiments of Woolley, Fekete and Little (1940, 1945); in a strain (CE) of inbred mice, either ovariectomy of females or castration of males shortly after birth gave 100 per cent yields of adrenocortical tumours which metastasized and were transplantable. Nothing extrinsic is administered to the mice and the tumour arises in a suitable genetic milieu by hormonal imbalance probably occasioned by adrenal hyperactivity in compensation for the absence of the gonads. Rhoads, Dobriner and their co-workers (1947) have suggested that adrenocortical hormone production and metabolism are very frequently disturbed in neoplastic disease. They have isolated from the ketonic fractions of human urines some 42 compounds, all steroids, of which at least 27 have been identified; one of these compounds, 3α-hydroxy-5-*iso*androst-9:11-ene-17-one (XV), appears to be associated with cancer. In Dr. Dobriner's words "a fair statement would seem to be that this compound is very significant but not wholly specific for cancer"; from the chemical point of view there can be no doubt that it is derived by dehydration of one of the 11β-hydroxy-sterolds characteristic of the adrenal cortex, and which are subject to

extremely ready loss of the 11β-hydroxyl group as a molecule of water (Shoppee, 1940). There is also the recent evidence (Hench, 1948) that Kendall's Compound E, or Reichstein's Substance F, *commonly known as cortisone (XVI), is highly* effective in the treatment of rheumatoid arthritis, and the suggestion has been made that steroid hormones may be *implicated quite generally in pathological conditions.* These matters are to be further discussed by Dr. Dobriner and Dr. Burchenal.



(XV)



(XVI)

Since anti-tumour activities of steroids are to be discussed by Dr. Stock, it will be sufficient to mention the suggestion of Lipschutz (1948) that steroid balance may be an auto-defensive anti-tumoural device, and to refer to the work of Professor Huggins on the effect of androgen withdrawal and the administration of œstrogen in prostatic carcinoma.

A brief reference to growth inhibition is desirable; it has been shown by Haddow (1937 *et seq.*) that there is some correspondence between the growth inhibiting property and the carcinogenic activity of various compounds. These observations relate to compounds of various chemical types, such as aromatic hydrocarbons, aminostilbenes, and both aliphatic and aromatic nitrogen mustards, but not to steroids; it is therefore of interest to recall the report of Heilman and Kendall (1944) on the effect of cortisone in inhibiting the growth of lymphosarcomata. There seems to be a considerable field of work here in the systematic examination of steroids for effects of this kind.

The work at the Royal Cancer Hospital on the inhibition of established tumours suggests that the primary effects of quite diverse chemical compounds may be fundamentally

similar and that inhibitory effects result from cross-linking of the protein fibres of the chromosomes, whilst the later appearance of tumours arises from the subsequent genetic change. In this connexion it is to be recollected that the 11-oxygen steroids of the adrenal cortex, e.g. cortisone, are considered to produce their biological effects by influencing protein metabolism (Long, 1942).

To a chemist, perhaps the most striking difference in the production of tumours by a so-called chemical carcinogen (e.g. hydrocarbons, heterocyclic aromatic compounds, azo-dyes, aminostilbenes, or nitrogen mustards) on the one hand, and by a virus (e.g. the Rous agent) on the other, is the time factor required; months are needed by the chemical carcinogens, but practically no time at all by the virus. The Rous agent appears from the available evidence to be a protein; that it is able to induce the change from normal cell to malignant cell at once suggests that this change involves protein synthesis.

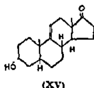
Haddow (1944, 1947) has suggested that a virus may possibly function as a substitute plasmagene: so that in an idealized model we have to envisage a bimolecular, cytochemical reaction between two proteins, the chromosome fibre and the substitute plasmagene, whereas if we introduce a carcinogen, e.g. methylcholanthrene, three chemical entities, chromosome, plasmagene, and carcinogen are concerned. The probability of a termolecular reaction is however about 1,000 times less than that of a bimolecular reaction, and to achieve the same reaction rate would require a very much lower termolecular activation energy.

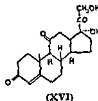
A reaction rate k may be expressed by the relation

$$k \propto e^{\frac{-\Delta H}{RT}} \cdot e^{\frac{\Delta S}{R}}$$

where the $-\Delta H$ exponent relates to the heat of the reaction and the ΔS exponent relates to entropy. This latter is concerned with molecular configuration; if therefore a biological reaction requires molecular conformation of the

extremely ready loss of the 11β-hydroxyl group as a molecule of water (Shoppee, 1940). There is also the recent evidence (Hench, 1948) that Kendall's Compound E, or Reichstein's Substance F, commonly known as cortisone (XVI), is highly effective in the treatment of rheumatoid arthritis, and the suggestion has been made that steroid hormones may be implicated quite generally in pathological conditions. These matters are to be further discussed by Dr. Dobriner and Dr. Burchenal.


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DISCUSSION

[illegible]

produced in the organism. Dr. Boyland was the first to investigate the metabolism of this type of compound Dr. Boyland and later we ourselves were able to show that during the metabolism of the carcinogens, hydroxylated derivatives are formed which are not carcinogenic. The cholesterol metabolites Dr. Shoppee mentioned are also hydroxylated compounds.

[illegible]

hormones.

HUGGINS What bonds do you get between steroids and proteins?

SHOPPEE· That is a very difficult question. The most obvious answer is that the hydroxyl group at position 3 which occurs in all natural steroids and which is known, for example, to tend to form double molecules, seems to be implicated. As a matter of practical interest, it is extremely difficult to get a 3-hydroxyl steroid really free from [illegible]

reactants involving loss of molecular degrees of freedom, this will lead to a low rate of reaction. It seems thus that the viruses, regarded as plasmagenes, may fit some part or parts of a normal chromosome with varying degrees of difficulty, and in consequence produce their effects with varying speeds. Steroids appear especially interesting because they *seem to stand intermediately between a virus*, such as the Rous agent, and the chemical carcinogens in regard to the speed with which their biological effects are produced.

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of substances could be differentiated by the ratio shown between the minimal effective dose applied directly to the vagina and the minimal effective systemic dose. A substance which has not got the two hydroxyl groups might conceivably be a pro-œstrogen, and might be converted into a biologically active œstrogen after injection.

WILLIAMS: Most of the pro-œstrogens are relatively inactive compounds.

gr
that it may be a π-complex.

PULLINGER: I understand that two-thirds of the œstrogen in the blood is protein-bound. Do the chemists know what that protein binding consists of?

SHOPPEE I don't think they do.

HIEGER: Those who have a chemical background often express a wistful longing for the isolation of polycyclic aromatic compounds in the body as an explanation for human cancer. They need not be altogether depressed at the failure to isolate such compounds, because these might only have a temporary existence in the body, and then be rapidly converted to some hydroxy detoxication compound. It is probable that attention may soon be focused on quite a different type

SHOPPEE *The dehydrogenations which have been accomplished* in the laboratory are all *in vitro* reactions at high temperature. The one attempt at an *in vivo* reaction by Butenandt and Dannenbaum with dehydronorcholene gave completely negative results

SOMMERVILLE: With regard to molecular configuration, it is obviously important to determine whether there are in fact inherent structural requirements related to biological action, for example, the distance between hydroxyl groups or hydrogen-bond-forming groups in the œstrogens

SHOPPEE: I couldn't agree more that configuration is important. In regard to this theory of the critical distance of the two hydroxyl groups, personally I don't like it. It's far too simple and specific. And you can

Nature, 161, 309).

FOLLEY: We should bear in mind Emmen's belief that there are œstrogens and pro-œstrogens, that is, compounds inactive themselves which can be converted into œstrogens in the body. These two groups

THE RÔLE OF DIETARY TRACE FACTORS IN HORMONE-INDUCED TISSUE GROWTH

R. HERTZ

THIS paper discusses certain studies on the necessity for particular dietary trace factors for the optimum tissue growth response to various steroid hormones, particularly the œstrogens. The general field of the dependence on nutritional factors for endogenous production of pituitary hormone and ovarian steroids will be omitted, because such dietary failures of endogenous hormone production in various nutritional deficiency states are highly non-specific effects. The discussion will relate more to the effects of such dietary trace factors on the tissue growth response to administered exogenous œstrogens when given in dosages which should yield extreme or maximum tissue-growth responses. Thus, the hormone in these studies that we will describe is not a limiting factor; it is the capacity of the tissue to increase its mass which will be the limiting factor. There were two dietary factors concerned: biotin and folic acid.

Biotin is one of the more recently identified factors of the B-complex on whose physiological significance we have really very little information. We first came to know of the existence of biotin from the fact that when animals are fed a high proportion of raw egg albumin in the diet they suffer a toxic syndrome which has been termed the "egg-white injury response." Rats which have been subjected to such an egg-white injury experiment are in extremely poor condition, with loss of hair and general debility; these rats die from biotin deficiency. The biotin deficiency comes about indirectly because the egg-white contains a specific protein factor which we term avidin. This avidin has the capacity to combine with the biotin in the diet to form an avidin-biotin

complex. This complex cannot be absorbed from the gastro-intestinal tract and passes out in the stool. Thus, these animals become biotin-deficient.

We have found that avidin is present, not only in the albumin of the hen's egg, but in the egg of practically every species of bird that we tested. We tested over twenty of them, and found that the presence of avidin was not related to the taxonomic origin of the bird. Avidin was also found in the gelatinous material surrounding the frog's egg; this material is the biological homologue of the albumin of the hen's egg. We also found it in turtle's eggs. It seemed to us that here was a substance which warranted further study.

In association with Dr. Fraps and others we undertook an analysis of the avidin content of the genital tract of the actively laying hen. We have used as an assay method for avidin a microbiological test employing a yeast organism which requires biotin for growth. Avidin will inhibit that growth, and addition of biotin will restore the previously inhibited growth.

The avidin content of the albumin-secreting portion of the genital tract is extremely high, and when one goes just 1 mm. beyond the albumin-secreting portion into the shell gland area one no longer finds avidin. It is found, however, in the mesentery which supplies the albumin-secreting portion of the oviduct. We find this distribution of avidin only if the hen is actively laying. If the hen stops laying, as a result of seasonal change or old age, then avidin is no longer found in the genital tract. There is no avidin in a sexually immature animal. This suggested to us that we might try to induce avidin formation in the three-week old chick. I will omit the experimental details of these studies which are published elsewhere.

Fig. 1 represents the genital tract of an untreated three-week old New Hampshire Red chick. This is the size of the structure obtained when one administers stilbœstrol for a six day period at a maximum dosage of 1 mg. daily, or any other œstrogen in a comparable dosage equivalent. We have



FIG 1 Genital tract of chick before and after œstrogen administration.

increase in the biotin content. In other words, the presence of avidin in the genital tract does not seem to alter materially the blood level of biotin under these experimental conditions. The total biotin activity is shown here, namely the capacity of the serum to support the growth of various biotin-requiring micro-organisms such as *Lactobacillus casei, Saccharomyces cerevisiæ*, and several others which were used quantitatively to check our results.

The situation becomes complicated since Trager has described an additional factor which he refers to as F.S.F. a fat-soluble factor, which is present in the serum of many species of animals and which has the capacity to replace biotin for the growth of many biotin-requiring organisms. It is not chemically biotin (it is fat soluble rather than water-soluble) and we refer to it as the "fat-soluble factor" or as "pseudobiotin."

Now, when œstrogens are given to a bird a very substantial degree of lipæmia occurs. We have found, associated with that lipæmia, a substantial increase in the amount of this F.S.F. material. In the case of the genital tract (Table II), there is a rather interesting situation. If biotin is administered to a previously untreated bird there follows some increase in the biotin content of the genital tract. Also, with

Table II

OVIDUCT BIOTIN LEVELS IN CHICKS

Treatment	No. of Chicks	Oviduct	
		Biotin	F S F
None	6	19 0	17·0
Stilbœstrol	8	25 0	33·0
Stilbœstrol, Progesterone	10	27 0	37 0
Biotin, Stilbœstrol	8	62 0	46·0
Biotin, Stilbœstrol, Progesterone	9	256 0	42·0
Biotin	5	57 8	37·4

used mainly stilbœstrol. The mucous membrane has become highly proliferated under œstrogen stimulation, and this is the origin of the avidin. Under stilbœstrol stimulation we get no avidin production whatsoever. However, if we add to the stilbœstrol 1 mg. of progesterone daily over the same experimental period, we find extremely high amounts of avidin in the albumin-secreting portion of the genital tract. This means that avidin production in the bird is brought about under conditions which are quite comparable to the luteal phase of the mammalian menstrual cycle, and that two hormones are involved in the induction of this specific secretory activity.

These observations made us feel that it would be worth studying what was happening in the blood under similar experimental conditions. In Table I there are some quantitative data concerning the biotin content of the serum of birds under various types of hormonal treatment. Again we used sexually immature birds, about three weeks of age. The biotin content of the untreated bird is 3·6 mμg. per ml. of plasma.

Table I

SERUM BIOTIN LEVELS IN CHICKS

Treatment	*Number of Chicks*	*Total Biotin Activity*	*Fat Soluble Factor*	*True Biotin Activity*
Untreated . .	15	3 6 (3 0–4 2)	3 8 (2 6–5 0)	1·3 (1·1–1 7)
Stilbœstrol . . .	12	22·0 (17–30)	15·0 (10–22)	8 8 (5·2–11 0)
Stilbœstrol and Progesterone .	8	24 0 (15·0–31)	11·0 (9 0–13·0)	10 0 (8 5–13·0)

Under stilbœstrol there is a very marked increase in biotin activity. If progesterone is added to stilbœstrol, and there is a reproduction of the endocrine condition in which the anti-biotin, avidin, is formed, we get no substantial further

increase in the biotin content. In other words, the presence *of avidin in the genital tract does not seem to alter materially* the blood level of biotin under these experimental conditions. The total biotin activity is shown here, namely the capacity of the serum to support the growth of various biotin-requiring micro-organisms such as *Lactobacillus casei*, *Saccharomyces cerevisiæ*, and several others which were used quantitatively to check our results.

The situation becomes complicated since Trager has described an additional factor which he refers to as F.S.F. a fat-soluble factor, which is present in the serum of many species of animals and which has the capacity to replace biotin for the growth of many biotin-requiring organisms. It is not chemically biotin (it is fat soluble rather than water-soluble) and we refer to it as the "fat-soluble factor" or as "pseudobiotin."

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Biotin, Stilbœstrol, Progesterone . .	9	256 0	42·0
Biotin	5	57 8	37·4

stilbœstrol alone, or with stilbœstrol plus progesterone, i.e. the condition under which we get avidin formation, the presence of the avidin does not attract from the circulation any of the biotin content of the serum and there is no substantial increase in biotin activity. However, if with stilbœstrol exogenous biotin is given at the rate of 1 mg. per day (an excess sufficient to flood the organism) a little more biotin can be got into the genital tract. Moreover, when stilbœstrol and progesterone are given and avidin is formed in the genital tract, exogenous biotin is concentrated in the genital tract in enormous proportions. In other words, this specific glyco-protein substance produced under specific hormonal stimulation, can, under these conditions, attract a specific dietary factor into the rapidly proliferating and secretory tissue. Just what that means we are not able to say at this time, but from the standpoint of the physiology of tissue growth it is extremely interesting.

Our next observations were on the effect of folic acid deficiency on œstrogen response in various species. When the chick is deficient in folic acid there is only a slight growth response in the genital tract, the tissues being increased three or four times rather than forty or fifty times, as they would be in animals on a complete diet. We used animals deficient in riboflavin and pyridoxine as controls against such factors as reduced food intake and general debility, and found that such debilitating deficiencies interfered only very slightly with the tissue growth response to maximum doses of œstrogen. We found also that we could get any degree of quantitative response between a complete failure of response and a maximum response as we restored increasing amounts of folic acid to the diet, and that there was, therefore, a definite quantitative interrelationship between the level of folic acid intake in the bird and the tissue growth response to œstrogens. It is interesting, however, that in the folic acid deficient animal treated with œstrogen, there is lipæmia and hypercalcæmia, metabolic evidence of the utilization of the œstrogen, but the tissue growth seems to be suppressed in some way.

The monkey failed to show an œstrogen response when it was on a folic acid deficient diet. In the folic acid deficient animal there is complete pallor of the perineum, no turgidity, very little cornification of the vaginal epithelium, and, on microscopic section, no proliferative response in the genital tract despite ten days of treatment with very high dosage of œstrogen, which in the case of the monkey was œstradiol benzoate. Dr. Hisaw stated recently (private communication) that he has been able to carry a monkey along on

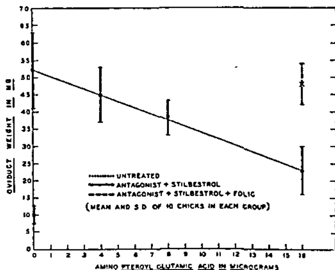


FIG. 2. Effect of aminopterin on œstrogen response in chick oviduct

œstrogen to get a proliferative endometrium; he then administered folic acid antagonists and precipitated menstruation by the interference of the folic acid antagonists with the utilization of œstrogens in the monkey. Dr. Goldsmith has shown that the folic acid antagonists could interfere with the frog oviduct response to œstrogens.

The chick oviduct is shown to be quantitatively inhibited in its œstrogen response in the presence of increasing amounts of folic acid antagonist (Fig. 2) A 48-hour chick oviduct

test was used which raised the weight of a day-old chick oviduct from 10 mg. to 50 mg. There is a quantitative interference with the response. The figure also shows the degree of restoration of that response when excessive amounts of folic acid are administered, even in the presence of this dosage of folic acid antagonist.

In the rat there is a little difficulty because a direct folic acid deficiency cannot be produced by dietary restriction because of the bacterial synthesis of folic acid in the intestinal tract. Therefore, folic acid antagonists have to be used. Eight of them have been used so far.

In the rat there is practically the same picture with respect to the uterine response, this time to œstradiol rather than to stilbœstrol. Ovariectomized sexually immature animals, whose uteri normally weigh 25–30 mg. each, average 80 mg. after 48 hours under œstrogen. Increasing dosage of folic acid antagonist brings this weight down almost to the level of the control. There is a slight residual tissue œdema which histologically appears to be simply an increase in interstitial fluid, thus giving a slight increase in weight. Histologically, the mitotic induction from the œstrogen is completely inhibited. Here also, when folic acid is administered in excessive dosage, provided the folic acid precedes the administration of the antagonist, we get a reversal of the inhibition. It is very essential that the folic acid be given before the administration of the antagonist, because if the antagonist, in this case aminopterin, be administered first it seems to become fixed in the tissue and cannot then be displaced. However, if the organism is previously flooded with excess folic acid the antagonist seems to have difficulty getting into its proper place. When the system is oversaturated with antagonist the restoration is poor, but just at the effective level there is practically complete restoration of response.

It should be emphasized that the tissue-growth responses to the steroid hormones are critically dependent and involved in a quantitative and specific way with dietary trace factors.

The important role of such a dietary trace factor as iodine has been appreciated for years and we know how critical such a minute trace factor is in thyroid function. We have come to appreciate more that these very potent dietary trace factors have a specific limiting role in the response to both endogenous and exogenous steroid substances. With the rapid development of the field of the antagonists to the B-complex factors we begin to have some tools for the control of hormone-induced tissue growth. From the standpoint of practical applicability we can say, from studies with breast cancer patients, that the available vitamin antagonists have such a narrow margin of safety that it is not feasible to try to employ this mechanism clinically. However, the biological principle is there, and perhaps with the development of more readily tolerable antagonists there may be some practical applications.

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DISCUSSION

BOYLAND: I worked for some years on the effect of sulphamethazine in causing hyperplasia of the testes of cockerels. A couple of years ago Dr. Hertz suggested that this might be due to the sulphamethazine having an anti-folic-acid-like action. We then tried to reverse this [illegible]

sulphamethazine.

[illegible]

purine antagonists would be worth study in this connection. Dr. [illegible]

[illegible]

diaminopurine. This may indicate a mechanism by which the œstrogen induces the tissue growth reaction. We feel that by studying the effects of various antagonistic systems we may find additional information on the specific biochemical systems involved in œstrogen-induced tissue growth.

STOCK: Is that a true reversal with adenine, or is it blocking?

[illegible]

HERTZ: No

BURCHENAL: I think the citrovorum factor would be very interesting. It does seem to have more effect in preventing the anti-leukæmic effect of the antifolics. The folic acid always has to be given ahead of time, just as in your experiments, whereas with the citrovorum factor you can get considerable effect if it is given at the same time, or even an hour afterwards.

HERTZ: I understand that it is much more effective in general against the toxic effects of the folic antagonists

BURCHENAL: It seems to be. On the other hand, if you have got very [illegible]

requires an entirely different set of nutritional factors. We have found that pyridoxine is critically required for androgen response in the seminal [illegible]

[illegible] specific responding end organ requires in higher amounts when responding to endogenous hormone.

Folley: We have done some work on the response of the mammary gland to œstrogen in relation to aminopterin, and we have not been able to show any interference either with the action of endogenous [illegible]

[illegible] you use to obtain the results you showed with the rat?

Hertz: An enormous dosage, 10 micrograms.

Folley: And how much aminopterin would suppress the effect of that?

Hertz: About 25 μg. would be sufficient to suppress completely the biological effect.

Your point about the general body growth is very pertinent. In studying the chick and the rat we used as controls animals with other [illegible]

[illegible] Cerecedo and others, does have deficient lactation, yet there is some [illegible]

Folley: Well, the mammary gland is certainly very sensitive to [illegible]

[illegible] per day and over that period the antagonist is so toxic that the rats die of anæmia.

What other folic acid antagonists have produced this inhibition of

Hertz: I didn't have time to include the data on 2,6-diaminopurine.

on the specific biochemical systems involved in ... growth.

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... on the same basis as the reversal

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... the folic acid

Another point is that avidin is a potent antibacterial agent and almost all organisms require biotin for growth. You can take a frog's egg out of a very polluted pond and culture the internal part of the egg and find it sterile.

BURCHENAL: You mean that besides binding with biotin, keeping it from being absorbed through the gut wall, avidin also keeps bacteria from using the biotin?

HERTZ: Yes, and that is the basis of our microbiological test for avidin. It interferes quantitatively with the growth of any organism which requires biotin, and you get quantitative reversal with excess of biotin

BEGG: Is there any avidin in the mammalian organism?

HERTZ: The homologue of the albumin-secreting portion of the hen's oviduct is the middle portion of the Fallopian tube We tested mucosal scrapings from the Fallopian tube of the cow, the sheep, the rabbit, guinea pig, rat, and mouse, and we always found a little biotin, but never any avidin. This might, however, be due to contamination with blood, or to technical difficulties.

CORNER: Did you consider the time of the cycle?

HERTZ. In the rabbit, we tested in the post-copulatory period, and in the other mammals in the post-ovulatory phase of the cycle.

FOLLEY: Is there any avidin in the albumin around the rabbit ovum?

HERTZ. We have looked for avidin in the mucosa which secretes the envelope and have failed to find it.

HERTZ: In most of our studies we are dealing with a 2-4 day test period. *In comb growth studies we deal with an eight-day period* in the chick, and that is feasible.

BURCHENAL: Could you not scale your dose down?

FOLLEY: We've done that, and 6 μg. a day seems to be satisfactory for our rats as far as toxicity is concerned, but then you don't get any effect on the mammary growth.

HERTZ: There are very marked differences in tissues. Dr. Nelson at Berkeley showed me her studies on the effects of folic acid antagonists on the young of pregnant rats, beginning the administration of antagonist early in the pregnancy. *She has obtained extreme abnormalities* [illegible] development, fetuses which are some three to four times [illegible]

tissues.

WILLIAMS. Has anyone tried to apply those substances locally, intravaginally?

HERTZ: I don't know of any studies. It should be feasible.

WILLIAMS. Do chickens have corpora lutea, and do they produce progesterone?

HERTZ: Dr. Fraps at Beltsville has shown that a second steroid substance is required for ovulation in the bird, and when he gives [illegible] intravenously *in large dosage during the pre-ovulatory* [illegible]

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the development of lymphoid tumours in mice yielded no uniform results.

The present experiments, which in part had originally been carried out in 1940 and 1941 and described by Dmochowski and Horning (1947), were undertaken to investigate the influence of œstrone alone and œstrone combined with castration on the lymphoid tissues of male mice of two partially inbred but non-homozygous, the so-called Hunt or "H" and MRC×Hunt or "R×H," stocks. The incidence of lymphoid tumours in female mice of these stocks is less than 1 per cent. The male mice of each stock were divided into three groups: those of the first were castrated and then painted with œstrone; those of the second were painted but not castrated; those of the third served as controls. Mice were castrated when three to four weeks old. Keto-hydroxy-œstrone in a 0·01 per cent solution in chloroform was applied twice weekly, starting one week after the operation, or at a similar age to the males which were painted only. Mice of the Hunt stock were painted for six months, and those of the "R×H" stock for four months.

The changes induced in the lymphoid tissues of the male mice of these two stocks were divided into three groups: those of the lymph nodes and other organs without changes in the thymus; those involving the thymus gland alone; those of the thymus, lymph nodes and other tissues and organs. The results are shown in Table I.

Table I

EFFECT OF ŒSTRONE AND ŒSTRONE PLUS CASTRATION ON LYMPHOID TISSUES OF MALE MICE OF "H" AND "R×H" STRAINS

Strain of Mice	*Experimental Procedure*	*Number of Mice Surviving*	*Mice with Lymphoid Changes Percentage*	*Mice with Thymus Changes Percentage*
"H"	Castration and Painting	65	58 5	18·4
	Painting	53	11 3	16 7
"R×H"	Castration and Painting	46	69 8	37·5
	Painting	48	18·8	33 3

THE INFLUENCE OF THE MALE AND FEMALE SEX HORMONES ON THE DEVELOPMENT OF LYMPHOID TUMOURS IN MICE

L. DMOCHOWSKI and E. S. HORNING

THE *influence of the female sex hormone on the development* of lymphoid tumours in mice was demonstrated by the observations of Mercier in 1938 and of Cole and Furth in 1941 that female mice of some strains have a higher incidence of lymphoid tumours than male mice of these strains. It was also demonstrated by the induction of these tumours in male mice with œstrogens (Lacassagne, 1937; Gardner, 1937), as well as by an increased incidence of lymphoid tumours as shown by Gardner, Kirschbaum and Strong in 1940; and by an earlier appearance of these tumours following œstrogen treatment (Shimkin, Grady and Andervont, 1941).

In connection with the significance attributed to the female sex hormone in the origin of lymphoid tumours in mice, several investigators carried out gonadectomy to ascertain its part in the development of these tumours. In some experiments such as those of Pybus and Miller in 1942, both ovariectomy and orchidectomy raised the incidence of lymphoid tumours. In other experiments, like those of Murphy (1944) and Law (1948) orchidectomy significantly altered the incidence, while ovariectomy had no effect. In yet another series of experiments, those of McEndy, Boon and Furth in 1942, orchidectomy failed to have an effect, and ovariectomy considerably lowered the incidence, while in the experiments of Gardner, Dougherty and Williams in 1944, and of Kirschbaum in 1944, gonadectomy had no significant influence on the appearance of lymphoid tumours in mice. This is by no means a full survey of the available literature, but it can be stated that the studies of the influence of gonadectomy on

and in the lymph nodes, as well as in other organs such as the spleen, liver, kidneys, or in the thymus alone. They consisted of enlargement of the thymus and/or of the lymph nodes with invasion of the surrounding tissues. Several tumours have been successfully transplanted, for many generations. There is no doubt about their truly malignant character. The first tumours were observed after six months, the latest after 15 months following the œstrone treatment. The difference between the present results and those of Gardner, Dougherty and Williams (1944) who observed no increase of tumours in their C_{57} black strain mice following œstrone treatment, may be due to sub-line differences. It should be mentioned here that Lacassagne considers œstrogens to be of greater

Table II
EFFECT OF ŒSTRONE PAINTING ON C_{57} BLACK MICE

	Control Mice			*Painted Mice*		
Sex	*Number of Mice*	*Number of Tumours*	*Incidence Percentage*	*Number of Mice*	*Number of Tumours*	*Incidence Percentage*
F	178	24	13 5	45	6	13 3
M	126	3	2 3	40	5	12 5

importance than heredity in the development of lymphoid tumours, while Gardner and his collaborators are of the opinion that the influence of sex hormones is conditioned by the genetic factor. We share this latter opinion. The genetic differences may have been responsible for the difference between the results of œstrone treatment of C_{57} black mice by Gardner and those of the present experiments.

Although no attempt was made in the present studies to assess the gradual changes in the lymphoid tissues under the influence of hormone treatment, there were indications that the amount of lymphoid tissue was decreased before the appearance of malignant changes, because in mice which died during the course of treatment, a decrease in the size of the lymph nodes and sometimes of the thymus and of the spleen

As can be seen from Table I, œstrone painting alone induces changes in the lymphoid tissues in the males of both stocks. The incidence of these changes was higher in the castrated and painted male mice. No changes were observed in the lymphoid tissues of 84 control mice. The changes in lymphoid tissues varied from hyperplasia to enlargement with invasion of the surrounding tissues. The earliest changes appeared after three months, the latest after twelve months from the beginning of the treatment.

Change in the thymus gland comprised enlargement of the gland, which consisted of enlarged cells with numerous mitoses, and was usually combined with invasive growth into the lungs, intercostal muscles, costal cartilages, pericardium, arteries, and into the trachea and diaphragm. All these changes were frequently accompanied by enlargement of the axillary, cervical, inguinal and mesenteric lymph nodes, combined with infiltration of the liver and kidneys, and with enlargement of the spleen. In some animals, however, the thymus was involuted. No changes were found in the blood of these animals.

It was not possible to establish whether these changes were of a truly malignant character because attempts to transplant the enlarged lymph nodes and the thymus gland were unsuccessful, possibly because the two stocks were not homozygous.

These experiments were followed up by another study of the influence of œstrone painting on males of a sub-line of C_{57} black low-cancer strain. They were painted, when four weeks old, with keto-hydroxy-œstrone in a 0·2 per cent solution in alcohol every other day for four months. The results are shown in Table II. As can be seen from the table, both male and female control mice develop lymphoid tissue tumours at an approximately equal average age of 12·5 months, varying from 5–22 months, although female mice show a higher incidence. The incidence of lymphoid tumours in male and female mice was approximately equal following œstrone treatment. The changes took place in the thymus

with similar invasive tendencies, and in a second rat we found a local malignant cystic tumour.

The incidence there is only about 2 in 100 in steroid-treated rats and none in control rats, so statistically it doesn't mean very much. The tumours occurred after two or three weeks of rather intensive therapy, so the time intervals are extremely small and it is highly unlikely that they are induced by testosterone. If anyone has any information on thymus tumours in rats I would be most pleased to have it. I wrote to Ingle and he could only give references to about five of them, and all of them had been associated with the administration of steroid hormones.

DMOCHOWSKI: It seems to me that too little attention has been paid to the genetic background of these changes. In the case of the mammary factor, we know now that there is a so-called inherited hormonal influence which is quite different from the hormonal factor which we have learned to know. Whether the same type of inherited hormonal influence may be responsible for the variable results of gonadectomy, and the fact that some mice give a higher incidence of lymphoid tumours than others I do not know. But I feel that geneticists may help the biologists and chemists a great deal.

HUGGINS: What was the incidence of these lymphatic changes in untreated animals?

DMOCHOWSKI: In the untreated H and R × H stocks, the incidence was below 1 per cent, so there was an amazing increase in these two stocks. The results may vary according to the kind of treatment; it is possible that the same amount of œstrogen supplied over a longer period may have a weaker effect of the thymus, for example over twelve months instead of three months. This is our observation and it agrees with that of Dr. Gardner and his colleagues.

HERTZ: In view of recent developments on the effects of steroids on connective tissue mesenchymal structures, might not the actual basis for the increased incidence be increased permeability of the ground substance by the lymphoid proliferating cells, rather than any direct effect on lymphoid tissue itself?

DMOCHOWSKI: Yes. It is very likely.

BEGG: In rats, while lymphoid elements themselves undergo dissolution with steroid hormone, the reticular material in the gland appears to undergo hyperplasia. It is difficult to follow because frequently you don't know whether you're seeing something which is hyperplastic and new or something which is uncovered because of disappearance of thymocytes. Phosphatase reaction gives a stronger reticular staining in the thymus after steroids than it does before.

DOBRINER: Can you stop œstrone application and still observe the same tissue changes?

DMOCHOWSKI: We did not stop the œstrone during the treatment, but the fact that quite a few of these tumours appeared within 12 months of the cessation of treatment indicates that once the process passes a certain threshold it becomes irreversible. Malignant changes appear eventually, but at the beginning there is, if anything, involution.

was noted. Furth (1946) suggested that the mode of action of œstrogens is to induce atrophy of lymphoid tissue, which is followed by regeneration. This suggestion and our own observations may have a bearing on the appearance of some tumours only 6–12 months after cessation of the treatment.

To conclude, it may be stated that the female hormone exerts a strong influence on the lymphoid tissues of male mice of a certain genetic background such as those of the strains examined, and the male hormone may have an inhibitory effect on the development of malignant changes of lymphoid tissues in these mice.

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DISCUSSION

STEROID HORMONES AND PROSTATIC CANCER IN MICE

E. S. HORNING

CANCERS of the breast, uterus and the prostate gland are of particular interest to the endocrinologist, not only because these are common sites of cancer in the human body, and are organs which are normally under endocrine control, but because the growth and behaviour of tumours derived from these particular organs can under certain conditions be influenced by means of hormonal therapy. Although, unfortunately, cancer of any of these organs cannot be permanently cured by this form of treatment, the most encouraging results have been demonstrated by Huggins and his school on the control of prostatic cancer in man by the application of anti-androgenic measures. It was not until the results of the investigations of Huggins and his co-workers (1941, 1945) were published that the influence of steroid hormones in the behaviour of prostatic cancer in man was fully appreciated.

In order to determine the effects of endocrine treatment on prostatic cancer in laboratory animals, it was first necessary to have at our disposal a transplantable glandular carcinoma of the prostate in pure line mice, similar histologically to those tumours of the prostatic gland which develop spontaneously in man. I propose to describe briefly the technique of subcutaneous homologous grafting by which these mouse prostatic tumours were obtained, and secondly to discuss the factors involved in obtaining successful grafts, before describing the influence of steroid hormones on the growth and behaviour of these transplanted tumours. I should like to make it clear at the outset that it is difficult to interpret these results obtained with mouse prostatic tumours in terms

DOBRINER: Have you any information on changes in size or morphology of the adrenals in your experiments?

DMOCHOWSKI: We have no information about the behaviour of the adrenals. It would be a very interesting study because we do not know yet whether the action is direct, through the adrenals, or possibly even through the pituitary.

DOBRINER: It was very interesting to me that in the beginning you got a decrease in lymphatic tissue, a decrease similar to that described with adrenal hormones. Dr. Woolley has found that adrenal size increases after œstrogen treatment. It seems to me that this may be a compensatory alteration in hormone production by the adrenal. Dr. Huggins, do you think that the œstrogen influence on tumours in humans is direct or indirect?

HUGGINS: It is direct

BEGG: In the rat thymus involution after administration of steroid hormones occurs in adrenalectomized animals. As far as the adrenal changes in these animals are concerned, you do get dissociation—after testosterone propionate you get tremendous losses in cholesterol, but practically none in ascorbic acid.

HERTZ: Are œstrogens the only factors which will induce thymic atrophy in the adrenalectomized animal?

BEGG: All the steroid hormones will produce involution in the adrenalectomized animal.

DOBRINER: Do they not produce overall decrease in the size of the tissues? Is this specific for the thymus?

thymic involution to a degree, and get change in adrenal size as well.

DMOCHOWSKI: In connection with the involution of the thymus you mentioned, I can't understand why in some mice we get this marked enlargement of the thymus gland, and in other cases the thymus is just gone. Might it be an individual response through the adrenals in some of the mice?

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DMOCHOWSKI. Yes.

prostatic epithelium are obtained from either the dorsal or ventral prostates of either strain A or strain C_3H mice. Under a dissecting microscope the selected pieces of epithelium are impregnated with crystals of the carcinogen, which in this instance was 20-methylcholanthrene, and the whole is implanted subcutaneously with the aid of a Bashford transplanting needle into host mice of the same age, sex and strain. Previous experiments have shown that since this technique involves homologous grafting, the reaction between the host and the graft plays an important role. In mice, the survival of an homologous graft seems to be dependent on the use of a closely inbred strain, as stock mice of indeterminate ancestry will not readily tolerate grafts of this kind. More recent experiments have indicated that the age of the donor providing the graft, and the age of the host mouse into which the grafts are implanted have an important bearing on the problem of graft survival. Another important factor is rapid vascularization of the graft in the subcutaneous tissues of the host animal.

A number of control grafts of prostatic epithelium without the carcinogen were implanted under the skin of host mice of the same age, sex and strain; these isolated fragments of prostatic epithelium continue to secrete prostatic fluid for as long as six months, provided that the grafts become successfully vascularized. The distension of the alveoli with fluid occurs to a degree far in excess of what is typical of the normal gland *in situ*, and invariably leads to a pronounced condition of cystic dilatation. In both the control and experimental grafts there is evidence that the secretion is slowly dispersed and absorbed within the tissues of the host. It will be noted that the prostatic epithelium in the control grafts is not hyperplastic, and hence it is important to distinguish as clearly as possible between growth in the grafts containing the carcinogen—this growth being due primarily to a failure in the release of the secretion, since ducts are no longer present—and proliferation which might be due to the direct action of the carcinogen.

of human prostatic cancer—but nevertheless they are of interest when studying the origin and cause of cancer in general. These mice experiments have, as we shall see, brought to light some facts of interest, amongst which is that the differences in response to endocrine therapy can be correlated with the histological structure of the particular tumour.

The technique of rapidly inducing tumours from adult prostatic tissues (impregnated with a carcinogen prior to subcutaneous implantation into host mice) was described by Horning in 1946. Since then Pan and Gardner (1948) of Yale have succeeded, by using the same methods, in inducing adenocarcinomas from adult mouse uterine epithelia impregnated with 20-methylcholanthrene. Also Boland Hughes (1949) of Pennsylvania University has obtained transplantable carcinomas of the bladder and adrenal cortex from homologous grafts combined with a carcinogen.

The mouse prostate gland, like that of the human, arises as a series of outgrowths from the embryonic urethra near its point of origin from the urinary bladder. The mouse gland consists of paired anterior, ventral and dorsal lobes, together with a small median gland, which may be absent or considerably modified in certain strains of pure line mice. Unlike the human gland, the mouse prostate has no uterus masculinus, nor is it encapsulated. A difference also exists in the number of ducts which open independently into the urethra. Thus in the human there are as many as 32, whilst in the mouse there are only six The prostatic epithelium is almost identical in its histology with that of the human. Hyperplasia of the prostate gland, however, never occurs spontaneously in rodents, as in man, but a similar condition sometimes arises in mice following prolonged treatment with œstrogens, which induces a pronounced metaplasia of the prostatic epithelium in the dorsal and ventral lobes, involving as in man urethral obstruction, retention of urine and hydronephrosis.

The technique of subcutaneous homologous grafting of prostatic epithelium is relatively simple. Small strips of

prostatic epithelium are obtained from either the dorsal or ventral prostates of either strain A or strain C_3H mice. Under a dissecting microscope the selected pieces of epithelium are impregnated with crystals of the carcinogen, which in this instance was 20-methylcholanthrene, and the whole is implanted subcutaneously with the aid of a Bashford transplanting needle into host mice of the same age, sex and strain. Previous experiments have shown that since this technique involves homologous grafting, the reaction between the host and the graft plays an important role. In mice, the survival of an homologous graft seems to be dependent on the use of a closely inbred strain, as stock mice of indeterminate ancestry will not readily tolerate grafts of this kind. More recent experiments have indicated that the age of the donor providing the graft, and the age of the host mouse into which the grafts are implanted have an important bearing on the problem of graft survival. Another important factor is rapid vascularization of the graft in the subcutaneous tissues of the host animal.

A number of control grafts of prostatic epithelium without the carcinogen were implanted under the skin of host mice of the same age, sex and strain; these isolated fragments of prostatic epithelium continue to secrete prostatic fluid for as long as six months, provided that the grafts become successfully vascularized. The distension of the alveoli with fluid occurs to a degree far in excess of what is typical of the normal gland *in situ*, and invariably leads to a pronounced condition of cystic dilatation. In both the control and experimental grafts there is evidence that the secretion is slowly dispersed and absorbed within the tissues of the host. It will be noted that the prostatic epithelium in the control grafts is not hyperplastic, and hence it is important to distinguish as clearly as possible between growth in the grafts containing the carcinogen—this growth being due primarily to a failure in the release of the secretion, since ducts are no longer present—and proliferation which might be due to the direct action of the carcinogen.

The neoplastic changes which occur in grafts implanted with the carcinogen will now be briefly described. Owing to the fact that the carcinogen is placed in direct contact with the living tissue, there is very little foreign body reaction or necrosis within the graft. Thus it is possible to study serial sections of the primary grafts, and to trace each invading clump of malignant cells back to the individual hyperplastic alveolus from which it has arisen.

Examination of prostatic grafts of various ages after implantation has shown conclusively that neoplasms are derived only from the alveolar epithelium which has entered the exhaustion phase of the secretory cycle. It is in these alveoli that hyperplastic epithelial changes are first seen, following a phase of mitosis, abnormal cell division and pycnosis. The hyperplasia is prominent in grafts four to five weeks old, giving an appearance to the epithelium which is closely similar to that seen in the human prostate during benign enlargement of the gland. In no single instance in any of the numerous grafts examined has the actively secreting epithelium lining the distended alveoli been the focus of malignant change. It is therefore presumed that the non-secreting exhausted alveolar cells are more susceptible to the action of the carcinogen. The hyperplastic changes in the mouse prostate are invariably accompanied by a pronounced increase in the fibromuscular stroma, and in some implants there is a lymphocytic infiltration which varies considerably in different grafts of the same age. In grafts six to eight weeks after implantation, the alveoli contain patches of epithelium which are 10–25 cells in depth, with cellular proliferation predominant in the basal layers.

At this early stage it is possible to distinguish three distinct types of epithelial proliferation. By studying the cytology and mode of growth of these early invasive cells through later phases of development in older grafts, it was possible to predict the type of tumours which would have arisen subsequently. The tongue-like colony of early malignant cells of the Type A is typical of those tumours which finally developed into

secreting glandular carcinomas. The Type B proliferation leads to the formation of a squamous-cell carcinoma in which alveolar formation is preserved. The more uncommon variety is the Type C lesion which arises from the duct epithelium and is characterized by a stratified squamous metaplasia *in situ*. In some grafts two varieties, types A and C of malignant change, co-exist in a primary graft and subsequently the tumour becomes a squamous-cell carcinoma, which rapidly infiltrates the adenocarcinomatous areas until all trace of the glandular tumour is lost. Both the glandular and the squamous-cell carcinomas grew on transplantation, and grew as serial transplants for many generations.

The observation that the focus of malignant change is restricted to the epithelium of the alveoli in the exhaustion phase of the secretory cycle is of importance. It would appear that the non-secreting alveolar epithelium is more susceptible to the action of the carcinogen than when at the height of secretory activity. These findings are also of interest because they support Haddow's (1947) contention that chemical carcinogens act more readily on cells following depression of cellular activity.

In view of the fact that œstrogens rapidly inhibit the secretion of the prostatic epithelium, and induce squamous metaplasia of the rodent prostate gland *in situ*, a series of experiments was undertaken to determine the influence of stilbœstrol alone, and stilbœstrol combined with the carcinogen in prostatic grafts growing in strain C_3H and strain A mice. In order to compare the depressant action of the œstrogen with the contrary action of the male sex hormone, which stimulates secretion of the prostatic epithelium, a number of grafts were prepared with the carcinogen alone and also with the carcinogen in combination with stilbœstrol and testosterone propionate. The results of these experiments are set out in the table on p. 36.

It was of interest to note that eight out of the 13 prostatic tumours induced with the carcinogen alone were glandular carcinomas. The remainder were all squamous cell tumours.

On the other hand, 23 of the tumours induced with stilbœstrol combined with the carcinogen were squamous *cell carcinomas*, and the remaining three were spindle cell sarcomas. As the sarcomas were far advanced it was impossible to ascertain by histological examination whether the tumours arose from the stroma of the graft or from the connective tissues of the host-bearing animal. Only three tumours, none of which were glandular carcinomas, were induced with the male sex hormone combined with 20-methylcholanthrene. Two were squamous cell growths, the remaining lesion being a spindle-cell sarcoma. These results are of significance inasmuch as they show that the inhibiting action of stilbœstrol on the

Group	*Substances included with the graft*	*Strain*	*Total No bearing grafts*	*No. which developed tumours*
I	20-Methylcholanthrene	A and C_3H	35	13
II	Methylcholanthrene and Stilbœstrol	A and C_3H	3[illegible]	26
III	Methylcholanthrene and Testosterone propionate	A and C_3H	35	3

secretory *epithelial cells* appears to render them more susceptible to the action of the carcinogen. Assuming this to be the case, it would help to explain why the focus of malignant change is always restricted to the non-secreting epithelium.

The glandular carcinomas induced from prostatic grafts by 20-methylcholanthrene alone are similar in many respects to the uterine adenocarcinomas induced by Pan and Gardner (1948), who used a similar carcinogen and employed the same technique of tumour induction, except, however, that these prostatic tumours secrete an enormous amount of fluid. Histologically the primary glandular carcinomas consist of well differentiated alveoli lined by a low columnar epithelium actively engaged in secretion. Unfortunately these glandular carcinomas are apt to undergo squamous differentiation during serial transplantation. A few of these tumours underwent this change during their third passage, whilst into

exhibited no evidence of squamous differentiation until their tenth generation of serial transplants.

The influence of orchidectomy was determined on the growth and behaviour of these glandular carcinomas as well as on those glandular tumours which had undergone squamous differentiaton during serial transplantation. In every instance both types of tumours were transplanted into male mice of the same strain from which the primary grafts were derived. Every host mouse receiving these transplants was castrated before puberty, and was approximately four to six months of age at the time of transplantation. The following silhouette chart (Fig. 1) illustrates the influence of bilateral orchidectomy on the glandular carcinomas.

The control groups are shown on the left hand side, which shows the same tumour transplanted into intact normal mice, and it was clearly seen that the tumours grew rapidly during the first five weeks after transplantation, so rapidly in fact that several of the host bearing mice had to be killed owing to the size of the tumours. On the right the effect of bilateral orchidectomy is seen on this tumour. All the tumours regressed with the exception of two which grew at approximately the same rate as those in the controls. Testosterone propionate was administered to three host mice bearing rapidly regressing tumours, and in one instance the tumour resumed its normal rate of growth. Further experiments have shown that the influence of orchidectomy on the growth of these glandular carcinomas is extremely variable, and so is their response to treatment with the male sex hormone.

The next silhouette chart (Fig. 2) illustrates the effects of castration on the squamous cell growths, and it is of interest to note that in no single instance did any of these tumours show any marked inhibition of growth when compared with those growing in the intact mice of the control group. This demonstrates that withdrawal of androgens does not appear to influence prostatic tumours once they have undergone squamous differentiation. It is therefore possible

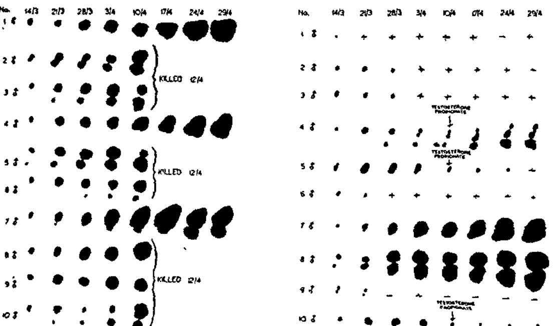

TRANSPLANTABLE GLANDULAR CELL CARCINOMA OF THE PROSTATE GROWING IN STRONG A MICE.
CONTROLS
CASTRATED
No.
14/3
21/3
28/3
3/4
10/4
17/4
24/4
29/4
KILLED 12/4
KILLED 12/4
KILLED 12/4
TESTOSTERONE PROPIONATE
TESTOSTERONE PROPIONATE
TESTOSTERONE PROPIONATE

FIG. 1.

to correlate the histological structure of these prostat
tumours with their response to androgens.

It is difficult to say why a secreting prostatic aden
carcinoma, once it has undergone squamous differentiatio

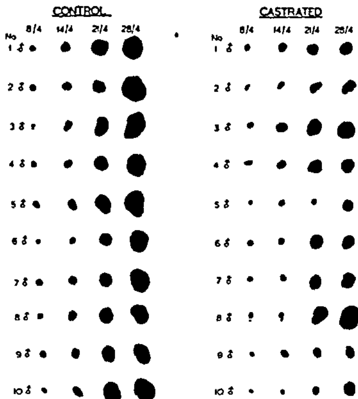


FIG. 2.

should cease to be dependent for sustained growth on a
adequate androgen level. It is still more difficult to unde
stand why these secreting adenocarcinomas should sudden
undergo differentiation into squamous-cell growths. Pulling

(1949) in a recent paper on "Functional Differentiation in Mammary Tumours" draws attention to the squamous differentiation in these prostatic tumours, and compares it to a similar phenomenon which occurs in the mammary glands of mice under experimental conditions. She contends that these changes might be due to an *intracellular œstrogen-like steroid.*

Summary

(1) The factors involved in obtaining successful subcutaneous homologous prostatic grafts are: (*a*) The use of a closely inbred strain of mice in which the grafts are more readily tolerated than they are in mice of indeterminate ancestry; (*b*) The rapid vascularization of the donor graft in the tissues of the host bearing animal; and (*c*) The age of the donor mouse providing the graft and the age of the host mouse into which the grafts are implanted; these factors have an important bearing on graft survival.

(2) Chemical carcinogens act more readily on cells following *depression of cellular activity. In grafts treated with the* carcinogen alone, the focus of malignant change has in every *single instance been restricted to the epithelium of alveoli* which have entered into the phase of secretory exhaustion.

(3) Tumours arise more rapidly and in greater numbers in prostatic grafts treated with the female sex hormone plus the carcinogen than they do in similar grafts which have been impregnated with the male sex hormone combined with the carcinogen, or in grafts treated with the carcinogen alone. It appears that stilbœstrol, by inhibiting secretion of the prostatic epithelial cells, renders them susceptible to the action of the carcinogen 20-methylcholanthrene.

(4) A number of secreting adenocarcinomas of the prostate, induced from homologous subcutaneous grafts of prostatic epithelium, regress when transplanted into mice castrated at puberty, and a smaller percentage of these tumours resume growth when the host mouse is treated with testosterone propionate. Tumours which at the time of their induction were diagnosed as squamous growths, or glandular carcinomas

which had undergone spontaneous squamous differentiation during serial transplanation, exhibit no appreciable response to this form of therapy. These results indicate that only glandular-cell carcinomas, and not squamous carcinomas, are dependent for their sustained growth on an adequate androgen level. This further suggests that the differences in response of the prostatic carcinomas in mice to hormonal therapy can be correlated with the histological structure of the particular tumour.

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DISCUSSION

HUGGINS: What little we know of cancer of the prostate has been learned almost entirely from human beings, there having been no [illegible]

with no carcinoma, and others in the "exhaustion phase," developing carcinoma. I would place a little different interpretation on that. I have come to believe that the presence of tall cylindrical prostatic epithelium in an organism signifies that androgen is present in an [illegible]

epithelium. I think that that is functionally better than this thin [illegible]

The picture is complicated by the fact that carcinogen plus stil-

of these types. Stra

has been treated by

glycolysis in large measure,

the tumour. The normal columnar epithelium has no aerobic glycolysis. So I tend to think that if œstrogen is in excess the cell is functionally quite different from when androgen is in excess.

I ... thinks that secretion develops ... ndly if he agrees that the ... ally active epithelium.

... early tumour formation ... carcinogen ... duces very ... raft which ... anges first ... of primary

grafts and to trace each invading group of malignant cells back to the individual hyperplastic alveolus from which it had arisen; in every ... epithelium was the focus of malig- ... All I can say is that these ... ibiting the secretion of the ... e susceptible to the action of the carcinogen; I can offer no other interpretation. It should also be borne in mind that many workers contend that chemical carcinogens act more readily on cells following depression of cellular activity, and that the depressant action of stilbœstrol in the prostatic epithelium in the mouse prostate has been the subject of an extensive cytological study.

Secondly, Dr. Huggins asks whether I think the alveolar epithelium in the exhaustive phase of the secretory cycle in the prostate gland is physiologically active. My answer is, yes. The same phenomenon occurs during the secretory cycle in the mammary gland. In both the prostate and mammary glands of rodents the exhaustion phase of secretion is followed by a recovery phase, in which the glandular epithelium gradually enters into the secretory cycle again. As far as I am aware, little is known concerning the factors controlling the cyclic rhythm of secretion in either the prostate or the mammary glands

ASTBURY: What is the significance of this aerobic glycolysis found in œstrogen-treated prostate?

HUGGINS: In general, cells with strong aerobic glycolysis are closer to the malignant, although not actually malignant, than cells without aerobic glycolysis.

BEGG: But there are really normal cells which do have high aerobic glycolysis.

HUGGINS: It can be debated whether that is not due to artifact.

[illegible]

glycolysis.

BEGG: What about papilloma and normal skin, Dr. Boyland? I believe [illegible]

[illegible]

Aerobic glycolysis is not specific, but it may be an indication.

BEGG: But if, for instance, you give testosterone to a rat you will find a change in the alkaline phosphatase activity of the kidney. There is a very definite metabolic change in the kidney as a result of giving the steroid hormone. I don't think anybody infers from that that the tissue change has been towards malignancy. I realize that this is a very debatable point, but I just wondered at Dr. Huggins using this as a criterion.

HUGGINS: I think Dr. Boyland has expressed my opinion much more [illegible]

[illegible]

origin.

HADDOW: Are your metabolic results consistent with Boyland's?

[illegible]

where you have both potentialities in the cells, for secretion and for [illegible]

[illegible]

who had escaped from anti-androgenic treatment with œstrogens. I would like to ask Dr. Huggins and the others here whether they have had any experience with progesterone.

HUGGINS: We did try progesterone in 1940, perhaps in insufficient amounts, but we observed no effect

HERTZ: Dr. Trunell used 200–250 mg. per day.

HADDOW: This might suggest that progesterone was effective only after œstrogens.

HERTZ: No, not that it is effective *only* after œstrogens But the patients he felt justified in studying were only those who had escaped from the beneficial effect of œstrogen and orchidectomy.

BURCHENAL: We have been interested in the problems of the resistance of tumours to various chemotherapeutic agents, and I was very

castration. Was there any morphological difference in those tumours from the ones that did respond well, and did you try transplanting those resistant tumours to other mice?

BEGG: It has been suggested that prostatic cancer patients who, after several years of favourable treatment, suddenly become refractory to treatment, may be getting androgens from some other source. Is it possible that even in the mammal there is a cellular adaptation to its environment, just as we know is found in bacteria and certain other organisms, which will change their enzyme systems, and their requirements for certain substances, depending on how long they have been

... that the malignant prostatic

enly

reek.

d to

orchidectomy were glandular carcinomas which had undergone squamous differentiation during transplantation. It was interesting also to observe that the glandular tumours which atrophied in response

to consist of

None of these

BEGG: Have you done any enzyme studies on these adenocarcinomas that have undergone squamous metaplasia? Acid or alkaline phosphatase?

HORNING No, so far I have not done so.

WILLIAMS I wasn't quite clear about the malignant change occurring in the exhaustion phase. Was the existence of secretory exhaustion deduced from cytological evidence, or just from the fact that there wasn't any secretion in the alveolus? Because, in the end, malignant change would prevent secretion.

HORNING: Whether or not any particular alveolus has entered into the exhaustive phase of the secretory cycle, is determined by the histological appearance of the glandular epithelium. The cytological

aspect of this problem has been studied extensively by many investigators.

HUGGINS: I want to point out that squamous carcinoma is one of the [illegible]

begun to grow?

HORNING: No, I transplanted these tumours into mice which had been castrated before puberty.

FOULDS: There is a considerable resemblance to some mammary tumours which I am down to talk about tomorrow. Some of those mammary tumours were transplantable only into female mice or into [illegible]

independent of the œstrogen, sometimes after one or two passages, [illegible]

Are these mammary tumours very slow growing?

FOULDS: Fairly.

HORNING: Prostatic tumours grow very rapidly. In some instances the glandular tumours following squamous differentiation, after they cease to become dependent upon androgens for sustained growth, grow extremely rapidly.

BEGG: In castrated animals are the grafts less vascular than in normal animals?

HORNING: These animals were not castrated.

CARCINOGENIC ACTIVITY OF STEROLS

I. HIEGER

In 1947 we published a paper the title of which was "Carcinogenic activity of preparation rich in cholesterol" and in 1949 a paper entitled "Carcinogenic activity of lipoid substances." Since only a few new facts are available at the moment, my remarks will therefore be more in the nature of a very b.. ef résumé of the present state of the investigation. It will hardly be necessary to say more than a word or two on the history of the subject. We would say that the development of synthetic cancer-producing compounds led to the successful search for naturally occurring carcinogens by Russian workers in 1937, when Schabad discovered the carcinogens present in the livers of human subjects who had died of cancer. The fahe livers of human subjects who had years by investigators its were followed up in the next few sources of carcinogen wer other countries, who showed that liver, in human tissue other be found also in non-cancerous rich (85 per cent) fractions of than liver, and in the cholesterol cholesterol was found to be rich tissues; finally, commercial producing agent. Just before no means inefficient cancer Shoppee, who had been with last stage of the research, suaded by Sir Ernest Kennaway Rothstein in Basel, was per forces working on the problem. come over to join our Beatty Institute, where he prepared He came to the Chester an elaborate series of processes. The very pure cholesterol fr gens of the 300 or so synthetic more powerful carcino produce cancer in 100 per cent of compounds available will Commercial cholesterol and the a very few months human tissues will induce 4–7 per ce. rich fraction of months. However, if the effective cancer in mice in

percentage calculation, that is, the incidence of cancer (sarcoma by subcutaneous injection) in the mice which survive the average latent period, the incidence then rises to 20 per cent. Shoppee's purest cholesterol produced one solitary sarcoma in 25 mice. This result very well illustrates the main difficulty of the work; the incidence is low, hence unless we make the quite gratuitous assumption that the mice are identically susceptible (and I fear that many investigators in cancer research *do* make this innocent assumption), work with large numbers of experimental animals is necessary. The interesting thing is that the non-cholesterol fraction from Shoppee's preparations, where the impurities were concentrated about ten times, did not produce a single tumour in 30 mice.

Furthermore, simple acetone crystallization of commercial cholesterol produced fractions, of which the least acetone soluble fraction gave tumours in four mice from a series of 20, while the untreated cholesterol gave a yield of two tumours in 30 mice.

In all, 27 sarcomas have been obtained from 450 mice injected with cholesterol not specially purified (the commercial product is labelled "re-crystallized"). In conversation with fellow workers the remark has frequently been made to me, that the impurities are responsible for the carcinogenicity of cholesterol. This assumption has one serious weakness; there is no evidence for it.

At any time, new data might alter one's perspective in this long term investigation, but at the present moment, if we might be so rash as to venture a prophecy, I would be inclined to say that fractionation of cholesterol will not yield any carcinogen with a potency as spectacular as the synthetic hydrocarbons; secondly, that individual susceptibility of the test animals is of the first importance; and thirdly, and this is the wildest guesswork, that the lipoidal focus attracts and kills mobile cells which liberate their fats as acids, and thus sets up a permanent subacute inflammatory region leading to neoplasia.

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hydrochloric acid in the body, but it doesn't burn holes in the body. We have to consider its dilution, the permanency of its location, and no doubt many other circumstances.

HADDOW: I take it that normally we probably have a considerable degree of interchange and conversion going on all the time, whereas

and progesterone are derived from cholesterol, but the site of the transformation (? the adrenal cortex) is not certain. I think it's

act that a low
cholesterol, for
where the sterol
content must be very low

naphthylamine. We injected these substances in the same sample of *olive oil, obtained from one source. We didn't get any tumours with* the β-naphthylamine but we got about 50 per cent of tumours with the α-naphthylamine. In control experiments, with the same olive oil we

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DISCUSSION

... to what ... the ... are ... here ... ited ... nge, but as to the identity of that substance, there is no information at all.

Gardner: How are the cholesterol, or the cholesterol derivatives, administered?

Hieger: A saturated solution in lard is injected subcutaneously. A ... added when ... naintain a

... llets? We ... e dilution experiments, and so far I have never seen a tumour around a cholesterol or a stilbœstrol-cholesterol pellet, although when injected in sesame oil, we have seen tumours.

Hieger: It is quite possible that the solvent plays a part in the formation of tumours. Lard itself gave no tumours when 350 control animals were injected. We were congratulating ourselves on this control experiment when an animal in the next batch of 50 suddenly ... 400

... 1 in 400.

Kellie: Is the so-called "commercial" cholesterol of animal origin?

Hieger: Yes, from the spinal cord.

Kellie: It has always impressed me that in a normal healthy person you get some 200–300 mg. per cent of cholesterol circulating through the blood stream and that most of the cells of the body must be in ... In con- ... per cent, ... mour for- ... seems to ... irculating ... when it is ... f but the ... s a lot of

hydrochloric acid in the body, but it doesn't burn holes in the body. We have to consider its dilution, the permanency of its location, and no doubt many other circumstances.

DOBRINER: How much did you give?

HIEGER: A total of about one-third of a gram was given. They were injected approximately every three weeks—just as often as was necessary to maintain the nodules under the skin.

HADDOW: I take it that normally we probably have a considerable degree of interchange and conversion going on all the time, whereas here we have a depot, a good fraction of which remains there without effective interchange. While Shoppee and Dobriner very much doubt the possibility of conversion of a naturally occurring steroid *in vivo* to an aromatic polycyclic hydrocarbon, nevertheless Cook still believes that the process is feasible. Is it not possible, Dr. Shoppee, that a conversion of that kind might be greatly facilitated in a local, highly artificial concentration of cholesterol to which, as Hieger said himself, is added such a factor as inflammation? Is it not possible that a sufficiently small degree of conversion, yielding possibly only a few gamma of a particular aromatic substance, might explain the result?

SHOPPEE: I think the only available evidence, from tracer work, is [illegible] transformation (? the adrenal cortex) is not certain. I think it's extremely difficult to express an opinion on your point

HIEGER: Against the degradation theory is the fact that a low carcinogenicity has been shown by fats other than cholesterol, for example, olive oil, sesame oil, lard, wheat-germ oil, where the sterol content must be very low.

[illegible] cancers simply because there is an abnormal milieu in the tissue. Dr. [illegible]

DOBRINER: I would like to mention an experiment that was done about 12 years ago and was never published. We were interested in the question of the carcinogenicity of aromatic amines. We obtained from one of the industrial plants very pure α-naphthylamine and β-naphthylamine. We injected these substances in the same sample of olive oil, obtained from one source. We didn't get any tumours with the β-naphthylamine but we got about 50 per cent of tumours with the α-naphthylamine. In control experiments, with the same olive oil we

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DISCUSSION

SHOPPEE. I don't think I can add anything very significant to what Dr. Hieger has already said. There is this serious difficulty of the difference in susceptibility of individuals, even when large batches are used. I think one must agree that the evidence is conclusive that there is some substance, or substances, present in this widely distributed material, cholesterol, which is able to bring about neoplastic change, but as to the identity of that substance, there is no information at all.

GARDNER: How are the cholesterol, or the cholesterol derivatives, administered?

GARDNER: Has it ever been administered in the form of pellets? We have used cholesterol pellets in connection with hormone dilution experiments, and so far I have never seen a tumour around a cholesterol or a stilbœstrol-cholesterol pellet, although when injected in sesame oil, we have seen tumours

HIEGER. It is quite possible that the solvent plays a part in the formation of tumours. Lard itself gave no tumours when 350 control animals were injected. We were congratulating ourselves on this

1 in 400.

KELLIE: Is the so-called "commercial" cholesterol of animal origin?

HIEGER: Yes, from the spinal cord.

administered in the form of a solution in lard.

HIEGER: One has to consider not only the substance itself but the conditions under which it is present in the tissues. There is a lot of

tration of such a carcinogenic hydrocarbon. Weigert's experiments showed that benzpyrene was very rapidly metabolized either in liver or in skin.

SOMMERVILLE: It wouldn't be necessary to argue the formation of [illegible] experiments might be due to the different rates of absorption.

BEGG: Have you any evidence that steroids are destroyed in the tissues?

FOLLEY: There is evidence, which came from experiments with labelled acetate, that cholesterol is turned over in the udder; the cholesterol may be synthesized, and at the same time may be degraded in the lactating udder.

obtained about 60 per cent tumours. We still can't explain why the olive oil with β-naphthylamine didn't give any tumours.

HADDOW: What kind of tumours were they?

DOBRINER They were sarcomas.

HORNING: Did you ever attempt to transplant these tumours?

DOBRINER: No, nothing more was done.

DMOCHOWSKI. I believe Dickens showed that a higher incidence of tumours can be obtained by using mouse fat as a solvent. In view of the small amounts of active principle in your extract, Dr. Hieger, might not a more favourable solvent be helpful in obtaining a higher incidence?

HIEGER. Such experiments are in progress.

DMOCHOWSKI: Have any experiments been done on the comparison of activity of extracts from cancerous livers and normal livers?

HIEGER Steiner found in his first experiments in 1943 that cancerous livers were more potent than non-cancer livers, but five years later he had to reverse his opinion, and found that the difference was as great as nine to one. This is further evidence that the sensitivity of the animal is extremely important.

DOBRINER: I think he did the second experiment on a different strain.

HIEGER: I think he used a number of strains in each case.

DOBRINER It seems to be somewhat unwarranted to conclude that if one gets carcinogenic material from such drastically treated material, that the substances were there originally. Would you have any comment?

Steiner does use a very drastic method for the saponification

made with extracts simply prepared with benzene?

MUHLBOCK. I can confirm what Dr. Gardner said. I have treated more than 2,000 mice with cholesterol-œstrone pellets, but I have never ... is no

but

nted

by sitosterol or stigmasterol.

BEGG: How long do you leave the pellets in?

MÜHLBOCK: One to two years.

BEGG: How big are these pellets?

MÜHLBOCK: One or two mg.

tumorigenic action of X-rays is apparently mediated through hormonal imbalances rather than by direct action upon the ovary (see Gardner, 1948*b*, for general statements and specific *schema*).

In our laboratory we have studied in considerable detail the responses of mice of seven different inbred strains, and of many hybrid groups, to prolonged treatment with œstrogenic hormone (Table I) (Gardner, 1948*a*).

Table I

TYPES OF TUMOURS APPEARING AMONG MICE OF DIFFERENT STRAINS WHEN SUBJECTED TO ŒSTROGENIC HORMONES*

Strain	*Mammary Tumours***	*Testicular Tumours*	*Lymphoid Tumours*	*Pituitary Tumours*	*Uterine Cervical Tumours*
A	+++	+++	−	−	++
C_3H	++++	−	+++	−	+++
CBA	+++	−	+++	−	+++
C_{57}	−	−	−	+++	+
JK	−	++	±	−	?
PM	−	−	++	±	++
$C_{12}I$	++	−	±	−	++

*Mammary tumours are the only tumours that occur spontaneously in mice of these strains and then only among female mice, whereas they appear in œstrogen-treated males Testicular or pituitary tumours have not been found in untreated controls Lymphoid tumours appear in a low percentage of mice of all strains and uterine cervical tumours only among mice of the PM stock

**++++ indicates a relatively high susceptibility and − no significant susceptibility to the tumours mentioned Reference + + +, etc , indicates intermediate degrees of susceptibility.

In our experience mice of two strains, C_{57} and PM, acquire pituitary tumours subsequent to œstrogen treatment. This response is particularly consistent in animals of the C_{57} strain. In other strains such tumours either have never appeared, or have appeared with extreme rarity. The tumours are usually firm and fleshy, although occasionally cystic, and hyperplastic. They may become very large, almost as large as a normal mouse's brain. They are benign in that they never metastasize or infiltrate the meninges or brain. Histologically they are composed of chromophobic cells and have been designated chromophobe adenomas. The

THE EFFECT OF STEROID HORMONES ON EXPERIMENTAL PITUITARY AND GONADAL TUMORIGENESIS

W. U. GARDNER

In the presentation to follow it will be assumed that at least some of the steroid hormones, notably the œstrogens, are carcinogenic or tumorigenic. This is an assumption that may have to be modified in the future. In their carcinogenic activities these substances may act quite indirectly and through the intermediation of some factors or influences that at the present time, for the want of a more specific designation, are termed "inherited," "strain limited," or "species limited" factors.

Species and strain limitations in tumorigenic responses are also prevalent to some degree in responses to carcinogenic hydrocarbons, as well as other carcinogenic substances that may be somewhat less specific in their action than are the œstrogenic steroids. It is not our purpose here, however, to argue the point of whether or not the steroid hormones are carcinogenic in the same sense as are the carcinogenic hydrocarbons. Until more is known of the mechanism whereby these substances modify cellular physiology the discussion must border on the metaphysical.

The presentation to follow will be concerned with modifications of the steroid and pituitary-gonadotrophic and possibly other hormonal balances upon the incidence of pituitary and gonadal tumours in experimental animals, primarily mice. In some instances the œstrogens evoke tumours; in other instances, prevent them. Evidence will also be presented indicating the tumorigenic action of pituitary gonadotrophic hormone. Furthermore, evidence will be presented to show that at least so far as ovarian tumours are concerned the

tumorigenic action of X-rays is apparently mediated through hormonal imbalances rather than by direct action upon the ovary (see Gardner, 1948*b*, for general statements and specific *schema*).

In our laboratory we have studied in considerable detail the responses of mice of seven different inbred strains, and of many hybrid groups, to prolonged treatment with œstrogenic hormone (Table I) (Gardner, 1948*a*).

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In our experience mice of two strains, C_{57} and PM, acquire pituitary tumours subsequent to œstrogen treatment. This response is particularly consistent in animals of the C_{57} strain. In other strains such tumours either have never appeared, or have appeared with extreme rarity. The tumours are usually firm and fleshy, although occasionally cystic, and hyperplastic. They may become very large, almost as large as a normal mouse's brain. They are benign in that they never metastasize or infiltrate the meninges or brain. Histologically they are composed of chromophobic cells and have been designated chromophobe adenomas. The

cells composing them, however, are unlike any cells in the normal pituitary.

The pituitary glands of female mice are larger than those of males. The pituitary glands of mice of all strains hypertrophy somewhat subsequent to œstrogen treatment, but usually the amount of hypertrophy is limited. The normal mouse's pituitary weighs approximately 2 mg. Pituitaries weighing in excess of 12 mg. have been arbitrarily called adenomas. In some instances these glands are probably hypertrophied rather than adenomatous. Occasionally adenomas are found in smaller glands.

Mice of the C_{57} strain transmit the tendency to pituitary tumours to their F_1 hybrids (Gardner, 1948*a*). Backcrosses of the F_1 hybrids to parental strains indicate that the tendency for pituitary tumours to appear is transmitted as a dominant character (Gardner, 1941, 1948*a*).

How œstrogen acts in producing the chromophobe adenomas in the pituitaries of suitable animals is unknown. In many ways the hypertrophy resulting is comparable to that of the thyroid following the administration of thiouracil. The gonadotrophin and growth-hormone output by the glands is decreased. Evidence of modification of the thyrotrophic and adrenocorticotrophic hormones is not available. It can only be stated that in suitable animals such tumours occur.

The tumours appear at earlier ages in male than in female mice and usually attain larger sizes (Gardner, 1941). The simultaneous injection of testosterone propionate inhibits the formation of pituitary tumours.

The pituitary tumours grow subsequent to transplantation into animals of the same strain. So far, however, only about 60 per cent of the tumours that have been transplanted have grown, and then in only about 20 per cent of the animals into which they were transplanted. Furthermore, they have grown only in œstrogen-treated animals. When transplanted subcutaneously the growths first appear after approximately nine months and then continue progressively. Most of the

hosts have had, at the time that the subcutaneous transplants were made, tumours in their own pituitary glands. After the transplants have started to grow they apparently grow progressively in the absence of further hormone treatment.

An attempt has been made to determine whether or not the original lesions of the pituitary are reversible subsequent to cessation of œstrogen treatment. It is impossible to determine whether or not a pituitary tumour is present until it has reached a considerable size, in which case the skull may be misshapen. Two groups of mice were each divided into a control and an experimental group. In the control group œstrogen treatment was continued. The animals were over 400 days of age. In the experimental group œstrogen treatment was discontinued. The mice survived for appreciable periods of time in both groups and had pituitary tumours of comparable sizes. From these observations it can be concluded that at least the pituitary tumours do not regress subsequent to the discontinuation of œstrogen treatment. It is quite possible that they continue to grow.

Ovarian Tumours

Ovarian tumours have been induced in experimental mice by two methods: (1) the intrasplenic transplantation of ovaries into castrated animals (Li and Gardner 1947*a*, 1947*b*, 1949) and (2) irradiation with adequate doses of X-rays (Furth and Butterworth, 1936). About three years ago the hypothesis was made that ovarian tumours in castrated animals bearing intrasplenic transplants arose because of the hormonal imbalance existing (Gardner, 1948*b*). The livers of the mice destroy œstrogens (Rush, in press). Zondek has first shown, about 17 years ago, that the rat's liver destroyed œstrogens. Subsequent to gonadectomy the amount of gonadotrophic hormone in the pituitary is increased (Engle, 1929, Evans and Simpson, 1929). Thus an ovary placed in the spleen, pancreas or other sites drained by the portal vein has its hormones destroyed by the liver before they reach the systemic circulation. In such animals

the pituitary gonadotrophin is elevated (Fig. 1). In such mice ovarian tumours of the granulosa cell type develop almost invariably. So far in our experience the tumours have not been strain-limited. Animals of six different inbred strains and many hybrid groups have acquired such tumours.

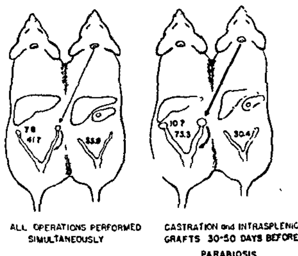


FIG. 1.
Miller a
number
weights
strated
gonadotrophins. This observation extends experiments undertaken by Dr. Li, in which he showed, by direct pituitary assay, that gonadotrophins were increased in animals with intrasplenic ovarian transplants.

The tumours first appear approximately six months after intrasplenic transplantation, although others appear after approximately 300 to 400 days.

Ovarian tumours have not appeared in *intrasplenic transplants in animals that have acquired adhesions of the* transplants of the parietal peritoneum, in animals that have

had one ovary or testis in place, or in animals that received œstradiol or stilbœstrol or testosterone propionate in adequate amounts (Li and Gardner, 1947a, 1947b, 1949).

The tumours are predominantly granulosa cell tumours although luteomas, which I believe should be called luteinized granulosa cell tumours, are not infrequent. Many of the tumours produce active substances of œstrogenic or androgenic type in such amount or of such quality that they by-pass the liver and produce effects upon the accessory reproductive organs of the host. If the tumours are permitted to grow to a large size the incidence of hepatic extension and pulmonary metastases becomes quite high. The tumours grow, in most instances, subsequent to transplantation into the subcutaneous tissues of hosts of the same strain. The percentage of takes and the rate of growth differ with different tumours.

Hypervolæmia was observed by Dr. Furth in animals bearing transplanted ovarian tumours (Furth and Sobel, 1947). A similar condition has been seen in our laboratory and has been studied, especially by Dr. Wolstenholme. The liver and adrenal glands may be increased in size several times by dilatation of the sinuses. The blood volume was greatly increased. Dr. Wolstenholme (1950) has demonstrated that this condition is reversible, subsequent to the removal of the tumour, and that the active substance will not by-pass a parabiotic union. The cause of hypervolæmia cannot be associated with the ability of a tumour to produce any of the known hormones. It is rarely found in animals having other than gonadal tumours.

One might ask why do not granulosa cell tumours occur more frequently in ageing animals? In advancing age the ova and follicles are depleted and presumably, also the hormone-producing capacity of the ovary. In such circumstances as after the menopause the ovary should be in an environment of augmented gonadotrophic hormone. In collaboration with Dr. Li (Li and Gardner, 1950) experiments were undertaken in which young ovaries were transplanted into old mice and old ovaries transplanted into old mice and

old ovaries were transplanted into young mice. Few tumours occurred in the intrasplenic transplant in the castrated mice of the first two groups but old ovaries transplanted into young mice became tumorous as frequently as did young ovaries transplanted into young mice. It thus seems that the ageing organism does not produce an environment as conducive to ovarian tumorigenesis as does the younger organism. Dr. Fern Smith in our laboratory (Smith and Gardner, 1949) has observed that the level of gonadotrophic hormone, as determined by the capacity of the pituitary to produce uterine stimulation in young assay animals, is decreased with advancing age after the age of 400–500 days. It is thus probable that gonadotrophic hormone production decreases with advancing age more or less in relation to the decrease in ovarian function.

Ovarian tumours also occur subsequent to X-irradiation. In preliminary experiments undertaken in collaboration with Dr. Li and Dr. Kaplan it was found that the injection of testosterone propionate in doses of 1·25 μg. weekly and *œstradiol benzoate prevented the appearance of such tumours.* More recent experiments, however, in which mice of the BC strain have been used, indicate the testosterone propionate in the amount administered does not prevent ovarian tumours; in fact the incidence has been somewhat higher than in the irradiated mice given sesame oil (Gardner, 1950) (Figs. 2 and 3). This, however, is in part due to the fact that testosterone propionate inhibits the leukæmogenic action of X-rays (Fig. 3). Œstradiol benzoate, however, completely prevented the appearance of ovarian tumours in irradiated animals, and also prevented the pre-tumourous changes observed subsequent to X-irradiation (Fig. 4). Experiments under way indicate that larger amounts of testosterone propionate will also prevent ovarian tumorigenesis in mice of the BC stock.

Testicular Tumours

Interstitial cell tumours of the testis appear in mice of the A strain subsequent to the injection of œstrone, œstradiol or

its esters, and stilbœstrol (Gardner, 1948*b*). Such observations have been made in several laboratories in England and

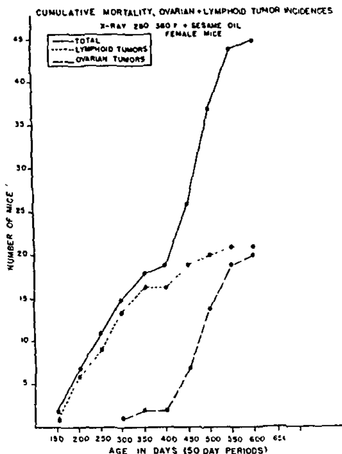


FIG. 2. Female mice of the B.C. stock (12th–15th generation of inbreeding) that received total body irradiation and weekly injections of 0·05 ml. of sesame oil. The number of lymphoid and ovarian granulosa cell tumours are indicated

America. One other strain, strain C, interestingly one of the parental strains from which Dr. Strong derived the original

A strain, also acquire such tumours subsequent to œstrogen treatment. In our experience testicular tumours have

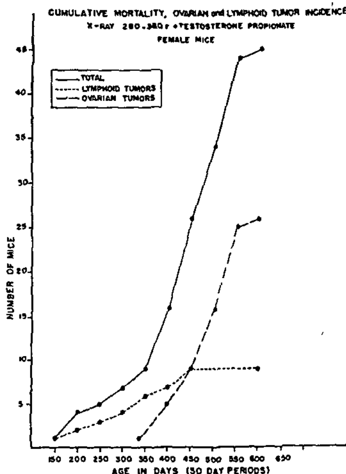
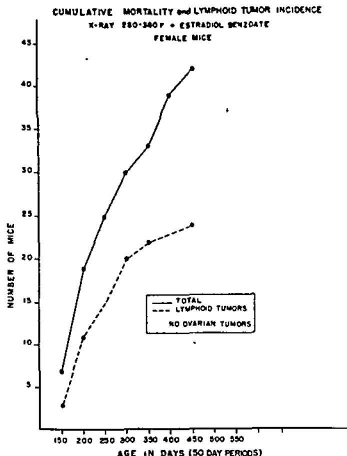


FIG. 3. Mice of the BC stock (12th–15th generation of inbreed-

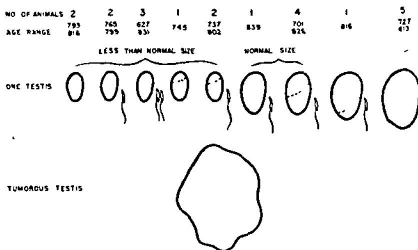
occurred with extreme rarity in œstrogen-treated mice of other than the A strain, or in untreated mice of any strain.

It has been assumed, but is as yet unproven, that in mice of the A strain the administration of œstrogenic hormones



results in an increase of pituitary luteinizing hormone or interstitial cell-stimulating hormone.

Recently it has been found that mice of one group, CC_1, which never acquire interstitial cell tumours subsequent to the injection of other œstrogens (Gardner, 1941) did so subsequent to prolonged treatment with tri-*p*-anisyl chloroethylene (TACE). Forty-six of 92 animals treated weekly with 50 or 100 µg. of this synthetic œstrogen had testicular interstitial cell tumours at death (Fig. 5). It is hoped that



the above observation may provide a method for determining how œstrogens may act in inciting interstitial cell tumours.

Summary

The tendency for pituitary tumours to occur in œstrogen-treated mice is strain-limited and is transmitted by both male and female mice of the C_{57} strain to their first generation hybrids. The tumours are not completely autonomous, in that they grow only, and then not consistently, in œstrogen-treated mice of the same strain. Prolonged quiescent periods have always preceded the growth of the tumours.

Ovarian grafts transplanted to sites drained by the portal circulation in castrated hosts give rise to granulosa cell tumours. These tumours can be prevented by the injection of œstradiol benzoate or testosterone propionate. Ovarian tumours occurring after irradiation (X-rays) can also be prevented by the injection of œstrogen. Ovarian tumours arising under both conditions are probably attributable to the gonadotrophic hormones produced.

Testicular interstitial cell tumours occur in œstrogen-treated mice of the A strain. Again, the tendency for such tumours is transmitted by both males and females to the first generation hybrids. A synthetic œstrogen, tri-p-anisyl chloroethylene, has induced tumours in mice ($C_{57}\times$CBA) previously not showing such tumours.

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DISCUSSION

PULLINGER. In the intrasplenic ovarian tumour experiment, can you transplant those tumours into intact animals? And in the pituitary tumour experiments, can you transplant the tumours into castrated animals? That would show complete independence of hormone in the two cases?

GARDNER. We have not attempted to transplant all of the ovarian tumours, but about 60 per cent of the tumours we have tried to transplant have grown subsequent to transplantation into intact animals. The incidence of take at the first transfer generation is variable. With some of the tumours there was evidence that progesterone increased the percentage of takes and the rate of growth. That has been published by Dr. Pan and Dr. Cliffton. After the first transfer, however, most of these tumours will grow in almost 100 per cent of the animals into which they are transplanted, whether male or female, or castrated.

I have not tabulated all the data, but the percentage of "takes" is high. As for the pituitary tumours, we have transplanted them into castrates, into normal males and females, and into œstrogen treated males and females. The only "takes" we have seen so far have been in the œstrogen treated animals, and even among those there has been a comparatively low incidence of tumours providing "takes," and a comparatively low number of "takes" with any one tumour transfer. We have not been able to carry any of them beyond the fourth transfer generation, that is, a span of six years.

MÜHLBOCK: In answer to Dr. Pullinger's question, I have been study- [illegible] but it requires a longer time in the castrated animals than in normal males.

[illegible] much of their specificity for the hormonal environment in which they develop. I think that the hormone environment which is best for their growth varies from tumour to tumour.

MÜHLBOCK: The principal cause for the development of all these [illegible]

the pituitary using immature animals. Dr. Fern Smith did some experiments on the age change in the pituitary, using both castrated and intact mice. She found that after 500 days the gonadotrophic hormone content of the pituitary decreased. She took pituitaries from mice from 300, 400, 500, 600 to 700 days, macerated them, injected them subcutaneously into infantile mice, and then weighed the uteri of the assay animals. Castrated immature animals were used as controls. The stimulation of the uterus is indirectly through the ovary and hence gonadotrophic hormone content is maximum at around 500 days, and decreases thereafter. Dr. Miller in our department put in parabiosis a pair of animals, one of which was castrated 30-50 days before the parabiotic union, and weighed the ovaries and the uteri of the intact parabiont after 14-21 days; the uterus weighed about 73·3 mg. Then he did the same thing with animals with an intrasplenic ovarian transplant, with the same effect. The intrasplenic ovarian transplant did not produce enough hormone to prevent the pituitary of the castrated parabiont with the intrasplenic ovarian graft from producing an abnormally large amount of gonadotrophin, stimulating the ovaries and hence the uteri of the intact parabiont. When Miller [illegible]

30–50 days the gonadotrophic hormone was high enough to produce a sudden effect in the intact parabiont. The same experiment is being done using animals with irradiated ovaries.

MUHLBOCK: There must be a difference between rats and mice, because we know that in rats there is an increasing amount of follicular [illegible]

[illegible]

very big ovaries but there are no tumours.

GARDNER. Have you put them in parabiosis with males?

MÜHLBOCK: Yes, with males and females of pure strain mice and hybrids The females which are very susceptible to œstrone production died, because of retention of the bladder and nephrosis indicating that there must be a great amount of œstrogenic hormone production. After parabiosis of several months, sections of the ovaries show the same

picture you find after the injection of the luteinizing hormone; very

it indicates th
treacherous to
not proven.
and FSH differentially.

MUHLBOCK The strain of mice is important for the development of

like to show. This is the hyp
[not reproduced]. Would you call that a tumour?

of the pitu
old mice, and we have studied a fairly large number of animals.

liver would inactivate
the livers of mice that
were subjected to chronic œstrogen treatment and castrated as well showed less activity, about 6 5 μg. per mg. per hour. I have often wondered whether the occasional mouse that shows these minimal ovarian changes might really have some deficiency in the œstrogen destroying mechanism.

DMOCHOWSKI In which strain did testosterone have an inhibitory effect on lymphoid tumours?

GARDNER: The data presented today were for mice of the BC strain, and that has since been confirmed in another series of animals. We find the same thing in both the males and females, but I showed only the data on the females. Earlier experiments on œstrogen treated ... gave similar results

mphoid tumours in

done only intact males and females.

DMOCHOWSKI. Is there a difference in the incidence between females and males?

GARDNER: Not in this strain. It is interesting, because in general, female mice show a higher incidence of leukæmia than male mice. The incidence in this strain is only about 3 per cent.

DMOCHOWSKI: Yet, in spite of that, testosterone seems to exert an inhibitory effect?

GARDNER: Yes, and the normal testis will largely prevent the increased incidence of leukæmia after irradiation. The incidence in the sesame oil treated X-rayed females was, as I recall, 53 per cent; the incidence in the sesame oil treated X-rayed males was 14 per cent, just the same as that of X-rayed females that got testosterone, so the testes are just as effective as 1·25 mg. testosterone propionate in [illegible]

[illegible]

tumours.

[illegible]

The incidence, with large doses of œstrogen, goes up to about 25 per cent, whereas in the control it was 1–3 per cent. If we [illegible]

[illegible]

KORTEWEG: You have shown that the possibility of growing pituitary tumours probably has something to do with hormones, and is also genetically determined. Did you find any correlation between this probable hormone factor and the genetically determined hormone [illegible]

[illegible]

GARDNER That's a very important point that I have thought about a great deal In my experience these tumours have occurred frequently in animals of what we call the BC, a low mammary tumour strain, [illegible]

[illegible]

If reciprocal hybridization is done, most of the animals will develop mammary tumours and pituitary tumours. So far as our own observations are concerned, I would think that any possible relationship with respect to the high incidence of pituitary and mammary tumours

after œstrogen treatment is just incidental. I have not, however, seen the tumours in the R-III animals, which are susceptible to mammary tumours.

KORTEWEG: Did your CBAs with tumours have the milk influence?

GARDNER: Yes. Our CBAs are just like the C_3Hs in this respect.

KORTEWEG. Then we can understand the fact that in the F_1 $C_{57} \times$ CBA, there are no mammary tumours, and in the reciprocals there are mammary tumours. Did you find that there was a positive correlation between the presence of the genetically determined hormonal factor and the appearance of tumours of the pituitary gland and of other organs?

GARDNER: Of course, we couldn't get results when we had the milk influence there because the mammary tumours developed so early that the animals didn't live long enough. I showed a slide [not reproduced] of one experiment in which we hybridized mice of the C_3H strain

PART II

THE MAMMARY GLAND

SOME EFFECTS OF STEROIDS ON THE MAMMARY GLAND

S. J. FOLLEY

I DO not work in the tumour field, but Professor Haddow asked me as an endocrinologist specially interested in lactation to contribute to this symposium in view of the outstanding importance of the mammary gland in cancer research. I thought that perhaps he would like me to give some general account of the effects of steroids on mammary growth, with special reference to the work that we have been doing in our laboratory at Reading.

Methods for Studying the Growth and Development of the Mammary Gland

First of all, I think one should emphasize the urgent need for some objective methods for studying the effect of hormones on mammary growth (see Richardson, 1947). For many years there has been a tendency among workers in this field to regard the study of only one mammary gland from each of a group of hormone-treated animals, either in the form of whole mounts or sections or sometimes both, as a sufficient basis for drawing rather far-reaching conclusions about the effect of various hormone treatments on the structure of the mammary gland. This is admittedly a time and labour saving procedure but its inadequacies may easily lead one astray. In the last few years we have, in our laboratory,

been trying to develop more objective methods for studying mammary growth, and our aim has been to make them as quantitative as possible. It should be emphasized, however, that this is proving rather difficult with the mammary gland. I shall begin by giving a brief account of the methods which we have been using, and which we hope continually to improve, for studying mammary development under experimental conditions. I hope that these methods may be of interest and perhaps of some use to cancer research workers.

In species such as the mouse, rat and monkey, in which the mammary glands are relatively flat (flat, that is, in all reproductive phases except late pregnancy and lactation) and can be stripped off in a thin sheet of connective tissue and made up into whole mounts, one objective measure of mammary growth is given by the rate of increase in the area of the mammary gland (bounded by the shortest line joining the periphery of the duct system). We have recently been carrying out studies involving measurements of the total area of all the mammary glands of the rat. Whole mounts are prepared, their images projected on to squared paper and their outlines traced. In this way the total mammary gland area for each animal can be computed. It should be emphasized that measurement of the mammary gland area, though affording an objective and quantitative method for studying the dynamics of mammary growth, only gives a general idea of the rate of growth of the duct system. It gives no information about morphological changes occurring within the periphery; it fails to detect increases in the degree of arborescence of the ducts, or the formation of new alveoli.

The most suitable and usual type of animal for studying experimental mammary growth is the immature animal which has been gonadectomized when sexually immature. Such an animal is, of course, still growing during the experimental period, and in studying the rate of increase of mammary area one has to take into account the concomitant increase in the body surface. This can be done by means of relative growth analysis (see Huxley and Teissier, 1938) which

enables a comparison to be made between the rate of growth of the mammary gland and that of the body surface.

The law of simple allometric growth of Huxley and Teissier, $y=bx^a$ (where y is the size of the organ under consideration, x the size of the reference organ—often the body as a whole—and b and *a* are constants), may be written $\log y = a \log x + \log b$. So that if the law is obeyed, a plot of log y against log x gives a straight line the slope of which is a, the equilibrium constant. If a is greater than 1, the organ is growing faster than the body (positive allometry), which is to be expected if it is a target organ responding to its specific hormone. If $a=1$, then the organ is merely growing at the same rate as the body as a whole (isometry), and any observed increase in the size of the organ must be discounted. This method of analysis was, I think, first applied to the mammary gland by Professor Zuckerman and myself in studies on the breast of the rhesus monkey some years ago (Folley, Guthkelch and Zuckerman, 1939). It has also been used in France by Dubois (1944) in some unfinished work which was unfortunately interrupted by his untimely death, and has been used in our laboratory more recently in studies on the mammary gland of the rat (Cowie, 1949).

Relative growth analysis is also very suitable for studying the dynamics of teat growth since the length of the teat can be readily measured and compared with the length of the body. As an example I might mention some work carried out in our laboratory some years ago, on the effect of androgens on the growth rate of the guinea-pig teat; this work showed that unsaturated androgens such as testosterone and androstenediol caused the teat to grow allometrically while saturated androgens such as *cis*-androsterone and dihydrotestosterone had no effect (Bottomley and Folley, 1938). Another example of the use of this method in teat growth studies is provided by our work on the normal and œstrogen-induced growth of the teat in the virgin female goat (Folley, Scott Watson and Bottomley, 1941). The teat of the normal virgin female goat begins to grow allometrically quite soon after

birth. The allometric phase is, however, eventually superseded by a period when the teat ceases to grow, and this occurs, curiously enough, just when the œstrous cycles of the goat's first breeding season begin. At the end of the breeding season, when the œstrous cycles cease, allometric growth, however, sets in once more. This curious phenomenon, which is the exact opposite of what one would expect at first sight, would clearly repay further investigation.

The mammary gland also grows by densification of the duct tree and develops by the differentiation of lobule-alveolar tissue. Therefore, in addition to the use of relative growth analysis in conjunction with measurements of mammary area, we have attempted to devise methods of obtaining objective measures of the increase in the arborescence of the mammary duct system and of the formation of alveoli. This problem has been attacked by devising a scoring system, applied to mammary gland whole mounts, which gives numerical scores susceptible of statistical analysis, thus providing a semi-quantitative if still somewhat subjective measurement of mammary development (Cowie and Folley, 1947). Dr. Dora Jacobsohn of Lund has also used our method, apparently with success (Jacobsohn, 1948).

The features which we score according to a pre-determined subjective scale are: the degree of arborescence of the duct system, the presence or otherwise of club-shaped end-buds (which usually take stain deeply and are considered to be a sign of active duct growth), and the status of the gland regarding the differentiation of side-buds and alveoli. The total whole mounts from each group of rats in an experiment are randomized and scored independently by two observers, the mean score for each feature being calculated for each group. The results can be submitted to an analysis of variance, thereby affording, we believe, a more valid picture of hormonal responses than if one gland only from each rat is studied microscopically in the usual way.

The third method I wish to describe is applicable to species that have "three-dimensional" mammary glands, i.e. udders

or breasts. It has been developed in collaboration with Mr. K. C. Richardson of the Anatomy Department, University College, London, who has been associated with us in these studies for a number of years. It has been used by us solely in studies of the goat udder, but in theory it could be used not only for the udder of the cow (though technical difficulties in working on such a relatively huge mass of tissue would be rather great) but presumably also for the human breast. In studying the structure of the caprine or bovine udder, it is very little use excising a small piece of tissue and fixing and sectioning it in the usual way, because it is apt to be full of milk, and as soon as the udder is cut the milk drains out and the whole organ collapses. The collapse of the distended alveoli precludes the possibility of getting a true picture of structural relationships. Moreover, in our experience, experimentally developed udders in the goat present a far from uniform histological picture so that a small piece of tissue is unlikely to be a representative sample.

In applying the method devised by Mr. Richardson, my colleague, Dr. A. T. Cowie, removes one half of the udder (one gland) from an anæsthetized goat—rather an exacting operation since the slightest puncture of the gland cistern is precluded otherwise the whole organ collapses—and as soon as it is taken off it is perfused with fixative under pressure. Later, in Mr. Richardson's laboratory, it is sliced in a horizontal plane into slices about 1·5 cm. thick, and fixing is continued. The slabs are finally infiltrated with collodion, and sections cut at 100 μ are stained and mounted as whole sections of the entire gland. The sections can be studied by suitable statistical sampling methods for "porosity" (the number of alveolar spaces per unit area, which is related to the mean alveolar diameter), and also examined microscopically.

With the help and advice of Mr. Richardson, an essentially similar method has also been applied by Dr. Cowie and Dr. M. H. I. Macaulay to the guinea-pig, which we have used as a pilot animal for the goat. The reasons for the choice of the

guinea-pig as pilot animal are (*a*) that its two mammary glands are "three-dimensional" of a form rather like udders in miniature, and (*b*) like the goat udder, the guinea-pig mammary gland responds with extensive alveolar development to the action of œstrogen alone. The whole animal is fixed under anæsthesia by perfusion through the aorta, the mammæ removed and serial sections prepared after collodion infiltration.

Mammogenic Effects of Œstrogen and Progesterone

In turning to consideration of our studies of the effects of steroids on mammary growth, it should first be emphasized that the common species of experimental animal can be divided into two main classes. First, there are those in which œstrogen alone evokes not only growth of the mammary ducts, but also considerable alveolar development. These include the guinea-pig, the monkey and the farm ruminants. The case of the rhesus monkey is of some interest. Earlier work (Folley *et al.*, 1939; Gardner, 1941) did not lead to unanimous agreement as to how far œstrogen alone would cause development of the mammary alveoli in the rhesus monkey, particularly the male. However, the recent valuable monograph of Speert (1948), who has had access to much more comprehensive experimental material than earlier workers, leaves no doubt that the monkey must be included in the species in which œstrogen alone causes extensive lobule-alveolar growth. As regards the ruminants, the cow and goat, in which we ourselves are mainly interested, numerous experiments on the hormonal induction of lactation (reviewed by Malpress, 1947) testify to the extensive alveolar development which can be obtained by œstrogen treatment. Fig. 1 shows a complete section at 100 μ. of one half of the udder of a virgin goat which had been given 1·0 mg. hexœstrol daily for 96 days, that is a period about equal to one half of the gestation period (Cowie, Folley and Richardson, unpublished). The extensive lobule-alveolar system developed by this treatment is shown very clearly. However, in considering

experiments which appear to demonstrate alveolar growth in response to œstrogen in the absence of the ovary, there is a complication to which I drew attention ten years ago (Folley, 1940), namely, the possibility that, under the influence of administered œstrogen, the adrenal cortex may secrete progesterone which, as is well known, is a mammogenic agent the action of which is closely related to the growth of alveoli. There seems to be no obvious way of overcoming this technical difficulty.

The second category, which includes the mouse, rat and rabbit, consists of forms in which, at any rate at physiological dosage levels, extensive alveolar growth requires progesterone as well as œstrogen. The position as far as the rabbit is concerned is very beautifully illustrated by the elegant work which Dr. Lyons has done on the optimal ratios of œstrogen and progesterone necessary for the experimental development of the lobule-alveolar system (Lyons and McGinty, 1941; Scharf and Lyons, 1941).

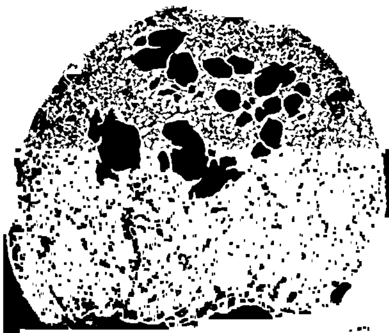
Goat. In our earlier studies on experimental udder growth in the goat, the mediocre lactational performances of the experimentally developed udders quite soon led us to suspect that although extensive udder growth regularly resulted from the administration of synthetic œstrogens alone, the alveolar tissue so obtained might not only be deficient in amount but might also exhibit morphological abnormalities, both of which features could perhaps be corrected if progesterone were administered as well. Indeed, Mixner and Turner (1943) in the United States have shown, in preliminary experiments with the goat, that udders grown with œstrogen tended to exhibit cystic alveoli in which papillomatous epithelial outgrowths were often seen, but if progesterone was given as well, the alveoli were smaller and the histology of the tissue more nearly normal In view of these considerations we have been investigating for some time the use of various ratios and absolute levels of œstrogen and progesterone for evoking artificial udder growth in spayed goats. So far only a brief, preliminary account of this work, which is still in progress,

has been published (Cowie, Folley, Malpress and Richardson, 1951).

In choosing the most likely gravimetric ratio for the best results we took into consideration the work of Dr. Lyons, just mentioned, in which he investigated the optimal ratios in the rabbit. On the basis of his results and using Dr. Corner's calculations of the output of progesterone by the rabbit's corpus luteum (Corner, 1937), and our own measurements of the size of the goat corpus luteum (Folley, Greenbaum and Roy, 1949), we calculated that daily doses of 40 mg. of progesterone and 1 mg. of hexœstrol were worth trying first in our search for an experimental régime capable of mimicking in the spayed goat the effect of pregnancy on the udder of the *normal female. The hormones were injected in oil daily* for 20 weeks (approximately the gestation period in the goat) and, since mammary growth largely takes place during the first half of pregnancy, some animals were injected for 10 weeks only.

In preliminary experiments the indications were that combined treatment with œstrogen and progesterone developed alveolar tissue histologically more normal than that obtained in response to œstrogen alone. However, subsequent experiments on a larger scale did not always bear out this indication though the results suggested that the combined treatment grew udders often capable of giving larger milk yields than udders grown with œstrogen alone. Alveolar "porosity" measurements made on sections prepared from udder halves removed from these animals at the peak of lactation were very variable and no statistically significant differences were ascribable to the different treatments.

For various reasons it seemed possible that the œstrogen doses used in these experiments were too high and that more *significant results might* be obtained with lower dosage levels. In this connection it may be noted that Clarke and Selye (1943) have reported that a given ratio of œstrogen and progesterone will evoke different responses in the vaginal epithelium of the rat according to the absolute level at which



[illegible]

By Mr R C Richardson

Photograph by Mr F J Pittock, F R P S.

[*To face page* 76



FIG. 2. Celloidin sections of mammary glands of ovariectomized virgin guinea-pigs. The sections, shown all at the same magnification, were cut at 30 μ. and stained with hæmatoxylin.

Top left	Untreated control
Top right	Guinea-pig receiving 0·1 mg. œstrone subcutaneously in oil daily for 68 days
Bottom left	Guinea-pig receiving 2·4 mg. progesterone in oil daily for 68 days
Bottom right	Guinea-pig receiving 0·1 mg. œstrone and 2·4 mg. progesterone subcutaneously in oil daily for 68 days

Photographs by [illegible]

the two hormones are given, vaginal cornification resulting at one level of dosage, and an entirely different response at a higher level. It seemed possible that the same might apply to growth responses of the mammary gland. We are therefore carrying out at the present time a preliminary experiment on four spayed goats in which a much lower dose of œstrogen (0·25 mg. daily) is being given, but at the same time we have raised the progesterone/œstrogen ratio to 400.1 by weight.

A very interesting effect has been observed in these latest experiments. The two goats receiving hexœstrol alone, towards the end of the injection period of 20 weeks developed remarkably large udders—as large as those of high-yielding pedigree goats in full milk. These udders were so turgid as to necessitate the initiation of milking before the end of the treatment period. By contrast the udder development in the two goats receiving œstrogen and progesterone was much less striking. At first sight it appears as if the high daily dose of progestero [illegible] the alveolar t [illegible] tain until the rather laborious histological studies on the udder halves removed at the peak of lactation are completed by Mr. Richardson. It is worth noting that Dr. Lyons found evidence of inhibition of alveolar growth by high progesterone/œstrogen ratios (just over 600:1) in his rabbits (Lyons and McGinty, 1951).

As regards the effect of steroids on the male mammary gland, though in many species, well known examples of which are the guinea-pig and the monkey, the male gland is equipotential with the female gland, our experience as well as that of others indicates that this is not so in the ruminant. In our experiments, castrated male goats subjected to prolonged œstrogen treatment have never shown any but the most

*[illegible]

restricted mammary development, the experimentally grown glands never extending beyond the base of the nipple, though containing some alveoli (Folley *et al.*, 1941). Dr. Malpress and I in similar unpublished experiments on steers, to which we gave œstrogen and progesterone, obtained very similar results (see Malpress, 1947).

Guinea-pig. *Our experiments on the hormonal induction* of udder growth and lactation in goats which are being carried out with a view to eventual practical application, are very expensive in animals and hormones. As was stated earlier we are at the same time investigating the optimal progesterone/œstrone ratios and levels for mammary alveolar development in a much less expensive pilot animal, the guinea-pig. In *experiments involving the* administration of various daily doses of progesterone and œstrone to spayed virgin guinea-pigs, set up according to a factorial design (for which we are indebted to Prof. C. W. Emmens), Dr. Cowie has found that in the guinea-pig, progesterone is necessary in addition to œstrogen for normal and full alveolar development. The type of result obtained is illustrated in Fig. 2. The dosage *ranges, given daily for 68 days (the gestation period) for the* best responses have been narrowed down to 50–100 μg. œstrone and 0·5–4·0 mg. progesterone and these ranges are being studied more closely. When the optimal progesterone/œstrone ratio for mammary growth in the guinea-pig has been worked out it is intended to try this on the goat.

Rat. I would like now to consider some experiments on the rat, which, along with the mouse, has been widely used for studying effects of steroids on mammary development. We have applied relative growth analysis to study the rate [illegible] normal animal and [illegible]

[illegible]

As [illegible] obtained from a plot of the mammary area against age in young animals is limited. Such curves, for instance, give no information about the age at which the ovary first begins to secrete enough œstrogen to change the isometrically growing

gland to an allometrically growing one. Using relative growth analysis, however, Dr. Cowie has shown that in female Norway rats from our colony the mammæ grow isometrically from birth until 21–22 days, when an allometric phase sets in, the constant α assuming a value of approximately 3. It therefore appears that the ovary first begins to secrete enough œstrogen to affect the growth of the mammary gland at 21–22 days. If the females are spayed at 21 days, isometric growth ($\alpha=1$) continues (Cowie, 1949).

Mrs. M. Silver, in our laboratory, has been investigating the effect on the equilibrium constant, α, of various dosages of œstrogen given to spayed females. The immediate object was to find out whether it is possible, by the administration of sufficient œstrogen, to induce faster relative mammary growth than is observed in normal females ($\alpha=3$). Some of the results obtained so far are shown in Fig. 3.

The results so far obtained show that with doses of 0·25 μg. œstradiol dipropionate every two days a value of α of about the same magnitude as that observed for the intact female is obtained. Increasing the dose to 1·0 μg. every other day did not increase α significantly. It therefore seems that excess of œstrogen does not cause the mammary ducts to grow much faster than the normal rate, which may represent about the maximum growth rate attainable. When we have determined the minimum dose of œstrogen which just gives an α value of 3, it may be that we would be justified in thinking that we have an upper limit for the œstrogen output of the rat ovary. In these experiments the value of α found for untreated, spayed controls was 1·3, which is somewhat higher than the value of unity required for isometric growth and which was obtained in previous experiments with rats from the same colony (Cowie, 1949). It is believed that this discrepancy is connected with the fact that in these experiments the animals were spayed at 10 days, whereas in the previous experiments of Dr Cowie they were spayed at 22 days, i.e. immediately before observations started. It may be that in the interval of 11 days between removal of the ovaries and

the start of observations in the present experiments, there was some activation of the adrenal cortex by a hypophysis released from the control of the ovary, resulting in the production of small amounts of mammogenic steroids, perhaps

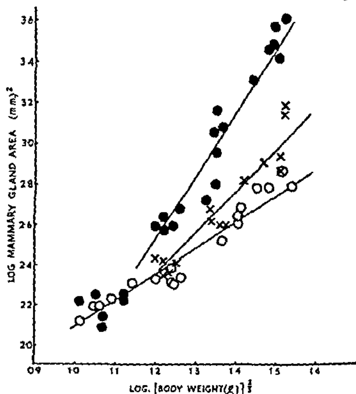


FIG. 3. Effect of œstrogen on the relative rate of increase in total mammary gland area in the young female rat.

● Normal intact females receiving 0·1 ml Arachis oil subcutaneously on alternate days.
○ Spayed females receiving 0 1 ml Arachis oil subcutaneously on alternate days.
× Spayed females receiving 0·05 μg. œstradiol dipropionate in 0·1 ml. Arachis oil subcutaneously on alternate days.

œstrogen. A more clear-cut, because longer term, example of this phenomenon was described by Fekete, Woolley and Little (1941). In support of this interpretation, Mrs. Silver

has found that in these early spayed animals the adrenals at autopsy were slightly heavier than normal for their age.

Similar results were obtained with male rats. Like the females, males gonadectomized at 10 days and killed at intervals from 21 days on, gave an α value somewhat greater than unity and the explanation is probably the same as for females. Administration of various doses of œstrogen gave much the same results as in the female rats.

This series of experiments has also included an investigation of the effect of aminopterin, a folic acid antagonist, on mammary growth as studied by this method of analysis. Hertz and Tullner (1949) have shown that administration of aminopterin to the spayed female rat virtually abolishes the uterine weight increase evoked by œstrogen treatment and have concluded that folic acid plays a part in this œstrogen-induced response. We therefore felt it to be of interest to see if there was any evidence that folic acid is concerned in mammary duct growth responses to œstrogen. However, we have so far been unable to demonstrate any inhibitory action of aminopterin on mammary growth, either in the normal intact female during the allometric phase or in the spayed animal receiving just sufficient œstradiol to cause allometric mammary growth.

Mammogenic Effects of Androgen

Rat. Coming now to the effects of androgens on the rat mammary gland as shown by relative growth analysis, it has been found that the mammary gland grows isometrically in intact males and that castration does not alter the α value, indicating that androgen at any rate at endogenous levels has little or no effect on the growth of the mammary ducts (Cowie, 1949). A study of whole mounts, however, illustrates the limitations of this analysis because whole mounts show that androgenic factors indeed have a remarkable effect on the mammary gland of the male rat In confirmation of the findings of earlier workers (e.g. Astwood, Geschickter and Rausch, 1937), Dr. Cowie has found that though the mammary

duct system in the young male rat remains restricted there is a striking development of clusters of alveoli at some time between 30 and 60 days. The mammary gland of the intact adult male rat thus has quite a characteristic structure; the duct system is restricted in extent but is covered with dense clusters of alveoli.

Rhesus Monkey. Just as in the case of the response of the mammary rudiment to œstrogen, so with androgen, species differences must be taken into consideration. This is exemplified by the case of the rhesus monkey. In experiments which I carried out with Dr. Van Wagenen at Yale some years ago we found that in the spayed female monkey androgen would only cause alveolar development if alveoli were already present (Van Wagenen and Folley, 1939). In these circumstances testosterone propionate was capable of causing the development of dense alveolar tissue while androsterone was inactive, recalling the effects, mentioned earlier, of these two androgens on the guinea-pig teat. Another observation which we made, confirming effects which Professor Zuckerman and I had described earlier (Folley *et al.*, 1939), was that papillomatous projections of the alveolar epithelium into the lumen were often present after androgen treatment in the monkey. In the monkey we have not succeeded in demonstrating growth of the duct system in response to androgen; the only noticeable effect was that the ducts were dilated with secretion. On the other hand it is well known that in the mouse administered androgen does cause duct growth (Van Heuverswyn, Folley and Gardner, 1939).

Since this is a conference on tumours, I feel I ought to conclude with a few words about cancer. In our work on the monkey mammary gland, Professor Zuckerman and I described some animals which had received regular treatment with natural œstrogens for periods ranging from over 365 to 938 days (Folley *et al.*, 1939). Although considered in relation to the normal life-span, this period of time is small in comparison with the period for which œstrogen is commonly administered to mice in order to induce mammary cancer,

it may be noted that we never saw any neoplastic or other abnormal conditions in these mammary glands when the animals were finally brought to autopsy. We felt at the time that this might provide some reassurance for the clinical use of œstrogen in women.

For the gift of material used in the unpublished experiments described in [illegible]
W [illegible]
C. [illegible]
and to Dr. J. H. Williams of the Lederle Laboratories Division, American Cyanamid Co. for aminopterin.

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DISCUSSION

LYONS: I do not think anybody would thank me for dragging in the hormones of the anterior pituitary at this time in connection with steroid function; but they do play very important roles in determining the response of the mammary gland to the ovarian steroids.

PULLINGER: I have often thought, like Dr Folley, that the mammary gland response might finally tell us the physiological level of œstrogen in these animals. I take it there is no other way of estimating what is the physiological amount of œstrogen circulating in the animal?

FOLLEY: No, I don't know of any. I believe Carl Hartmann has proposed a method of measuring œstrogen on a micro scale in blood, but I don't know that it has ever come into wide use. Perhaps Dr. Gardner could tell us more about it. It seems to me that this method, when we've got more data, might well give us some idea of the physiological output of the rat ovary, provided one can grow the gland faster experimentally than it grows normally. From our experiments, at the moment it looks as though the daily output is between 0·025 and 0·125 micrograms of œstradiol a day, which is rather small.

BEGG: Won't you eventually have to use adrenalectomized ovariectomized animals?

FOLLEY: I suppose we should, but it is very difficult to keep the adrenalectomized animals alive on an œstrogen regime.

BEGG: Have you used salt?

FOLLEY: Dr. Cowie didn't find salt very effective in our rats.

BEGG: How about salt and DCA?

COWIE: Then you would be adding a steroid which might affect the mammary growth.

FOLLEY: DCA, as Dr. Gardner and I showed years ago, does possess mammogenic activity. That was later confirmed by Speert in the monkey.

GARDNER: Did you use any animals, such as the dog, in which œstrogen had no effect on the growth of the mammary gland, or very very slight effect ?

FOLLEY: No, I didn't use those.

GARDNER: There is another class, in addition to the two classes you mentioned, in which œstrogens has a very limited effect. Dr. Trentin has recently found that with œstrogen plus progesterone the dogs' mammary glands do very well.

In this analysis of growth of the mammary gland I wondered about the extrapolation with increased doses of œstrogen. We have observed in our laboratory that large doses of œstrogen inhibit mammary growth, disproportionately to total body size. It inhibits general growth too, but if we give crude pituitary extract to keep body growth comparable to normal body growth, œstrogen still inhibits mammary growth. I don't know just where that would fall as far as dosage level is concerned.

I am sure that the dosages you were using here were much below that inhibitory level.

area of all mammary glands in each rat, and, for controls, using other rats. We don't use glands taken before treatment begins from the experimental animals for our controls. Judging by our curves, that

animal.

Boyland: Have you done any experiments with sheep? Sheep should be convenient animals because they are cheaper and more uniform than goats.

Folley. I don't think they're so much cheaper if they are pedigree animals

Sommerville: I think that Dr. Folley would agree that when trying to deduce endogenous physiological hormone production in studies in which the steroid hormones are administered, it would be wrong to neglect the possibility of shifts in the intermediary metabolism which might alter the whole picture. There is evidence for this in pregnancy where there may be an increase in progesterone secretion but, on the other hand, there may be shifts in the intermediary metabolism of the hormone reflected in urinary steroid excretion.

Folley. I think that's an interesting and important point and one that we obviously have to consider. I think that many of these conclusions are rather tentative and subject to considerations of this sort. Would you think that by giving very low physiological doses to spayed animals you may get shifts?

Sommerville: I should think it is less likely.

Folley: In our experiments the doses were of the order of 0 025 μg a day, and such factors should be at a minimum under these conditions.

Hertz Absorption may be a critical factor in your calculations comparing the biological effect of an injected dose and endogenous production.

FOLLEY: I agree that that is possible.

MUHLBOCK: In all your experiments the injections were started at the age of 20 days?

FOLLEY: Yes.

MUHLBOCK: Have you any experience with earlier treatment?

FOLLEY: No, we want to try that in due course.

MUHLBOCK: I have done some experiments to develop the mammary gland in mice in the early days, and there I found no growth. I began the treatment on the first day of life and I continued for 18–20 days.

PULLINGER: I agree with Dr. Muhlbock.

FOLLEY. We suspect that the mammary gland may not be responsive to œstrogen below a certain age. Dr. A. Reynaud of the Institut du Radium in Paris has been working on the effects on the fœtus of steroids administered to the mother, and he believes that the mammary gland is not responsive in late pregnancy. He wrote to us to ask if we had any evidence of what happens during the early period of lactation, from birth to 20 days, and we do intend investigating that.

HERTZ: There must be a marked species difference, because at birth in the human infant there is frequently stimulation.

FOLLEY Yes, I think Dr. Lyon's work on the hormonal basis of "witches milk" is interesting in this connection.

LYONS I think about two-thirds of all new-born infants of both sexes show mammary swelling due to growth of the parenchyma, and not simply to inflammation; and about two-thirds of these secrete milk or colostrum.

BUGG. Isn't the human fœtus biologically more advanced at birth than the rat or the monkey?

LYONS: I think the difference might be in the response of the fœtal mammary glands to the hormones of the placenta.

FOLLEY: Some goats have little udders at birth.

DOBRINER: I would like to ask Dr. Folley whether he has studied other hormones. He mentioned the corticoids. Have you tried the effect of any of the 11-oxygenated steroids on mammary growth?

FOLLEY: Not yet. Unfortunately, we haven't been able to get any cortisone so far. But Dr. Gardner and I showed some years ago that deoxycortone acetate causes mammary growth. We have investigated the effects of adrenalectomy, and concluded that in normal mammary growth the adrenal cortex doesn't seem to be important. In other words, one can by experimental means induce more or less the same degree and rate of mammary growth in adrenalectomized animals as in normal ones.

DOBRINER: The question of DCA is a very tricky one because one of the next intermediates in metabolism is progesterone. We have evidence that it is very easy to reduce the 21-hydroxyl.

LE RÔLE DES HORMONES STEROÏDES DANS LA CROISSANCE NORMALE ET PATHOLOGIQUE DE LA GLANDE MAMMAIRE

A. CHAMORRO

L'OBJET de cette communication ne sera pas, comme le titre pourrait le faire supposer, de présenter une mise au point de l'état actuel de nos connaissances sur les facteurs hormonaux qui déterminent la croissance normale de la glande mammaire ou qui interviennent dans sa pathologie. Tout dernièrement des mises au point ont été publiées par Folley (1947), sur le contrôle endocrinien du développement physiologique de la mamelle, par Nathanson (1947), sur la relation entre les hormones et la pathologie mammaire, et par Lacassagne (1948) sur les hormones et leurs relations avec le cancer et sur la position actuelle du problème de l'action cancérigène des substances œstrogènes (1949). La bibliographie sur les thèmes que nous allons traiter se trouve dans le snotes que, sur la glande mammaire, nous avons présentées depuis 1940 aux Sociétés de Biologie et d'Endocrinologie de Paris.

Nous présenterons une vue d'ensemble des recherches que, sur le rôle des hormones stéroïdes dans le développement normal et pathologique de la glande mammaire, nous avons réalisées au cours de ces dernières années. Nous rapporterons aussi des expériences encore inédites, et les premiers résultats d'autres en cours d'éxécution. Nous ferons, au préalable, une esquisse de l'évolution de nos connaissances sur le déterminisme hormonal de la croissance physiologique de la glande mammaire qui a passé par deux étapes: dans la première, l'ovaire a été considéré comme le centre trophique, dans la deuxième, l'hypophyse détient le rôle le plus important.

La Mamelle sous le Contrôle Hormonal de l'Ovaire

On sait que le développement et l'épanouissement physiologiques de la mamelle sont liés au sexe féminin, mais les données scientifiques sur la subordination de cette glande à la sécrétion interne de l'ovaire ont été seulement apportées au cours du dernier demi-siècle.

Le retentissement que l'extirpation des ovaires a sur la mamelle, a été signalé par Roberts dans son journal de voyage de Delhi à Bombay: des femmes ayant été castrées avant la puberté et examinées par lui à l'âge d'environ 25 ans, présentaient une aplasie de la glande mammaire et du mamelon ainsi qu'une atrophie des organes génitaux. Poth, en 1777, ayant extirpé à une femme les deux ovaires devenus tumoraux, observa que la menstruation cessa et que les seins, très développés, s'affaisèrent. Keppler (1890), ayant réalisé la castration de 46 femmes à cause de maladies gynécologiques, constata une diminution du volume des seins qui ressemblaient à ceux de l'homme, ainsi qu'une diminution de la pigmentation de l'aréole et du mamelon.

La première observation expérimentale de l'influence de l'ovaire sur la mamelle a été rapportée par Hegar en 1878. Cet auteur ayant extirpé un ovaire à une truie et les deux ovaires à une autre, constata que chez la première, les mamelles étaient toujours mieux développées que chez la deuxième. Cette constatation est confirmée par Halban (1900) chez le cobaye et par Foges (1905) chez la lapine impubère chez lesquels la castration empêchait le développement normal des mamelles. Les effets de l'extirpation des ovaires sont complétés par les expériences de greffe réalisées par Knauer, Halban et Steinach. Knauer (1899) réalise la transplantation d'ovaire chez la lapine. Dans le cas de transplantation autoplastique, la greffe prend et les mamelles se développent; dans le cas de transplantation homoplastique, la greffe ne prend pas, l'ovaire est résorbé et les mamelles s'atrophient. Halban (1900) chez le cobaye, constate que la greffe autoplastique d'ovaire provoque un développement

normal de la mammelle. Il admet que l'ovaire produit une substance spécifique nécessaire pour le développement et le maintien de la mamelle et considère l'ovaire comme son centre trophique. Steinach (1912), qui réalise l'implantation d'ovaire chez le cobaye mâle castré, signale que les mamelons, l'aréole et la glande mammaire prennent la forme et le volume de ceux de la femelle normale.

La dépendance de la mamelle à l'ovaire ayant été établie par les observations cliniques et expérimentales, Ancel et Bouin (1909–1911), dans leurs expériences chez la lapine en pseudo-gestation, mettent en lumière le rôle important joué par le corps jaune dans le développement des acini mammaires.

Après la constatation des effets de la castration et de la greffe d'ovaire, la troisième phase expérimentale, l'étude de l'action des extraits glandulaires, est réalisée par Fellner (1913), chez la lapine castrée à l'aide d'extrait ovarien et par Herrmann (1913), chez les lapins mâles ou femelles castrés par administration d'extrait de corps jaune. Ces auteurs constatèrent que ces extraits ovariens stimulent la mamelle. Allen et Doisy (1923) en injectant du liquide folliculaire ou de l'extrait de liquide folliculaire à la souris castrée, ont aussi stimulé la croissance de la glande mammaire.

L'isolement, l'identification chimique et la préparation à l'état pur des deux hormones stéroïdes de l'ovaire, ont permis aux expérimentateurs l'étude de la part qui revient à la folliculine et à la progestérone dans l'édification et les changements physiologiques du réseau mammaire. De nombreuses expériences ont établi que la folliculine développe les tubes mammaires et que l'action synergique de celle-ci et de la progestérone développe les acini. Les études histologiques de la glande mammaire de la femme ont révélé des changements cycliques de l'épithélium mammaire et leur parallélisme avec le cycle sexuel.

La Mamelle sous le Contrôle Hormonal de l'Hypophyse

Les observations de Cushing (1909) et de Aschner (1909)

sur l'atrophie de l'appareil génital consécutive à l'hypophysectomie, et celles de Zondek (1926), de Aschheim (1926) et de Smith (1926) sur l'action stimulante des implantats d'hypophyse sur l'ovaire, ramènent l'hypophyse au rôle dirigeant dans le système endocrinien. L'hypophyse contrôle l'ovaire et, à travers lui, la glande mammaire. Mais ce rôle de l'hypophyse ne se limite pas à l'action exercée par voie indirecte. En 1935, Selye, Collip et Thomson signalaient que l'administration d'œstrone à des rats femelles hypophysectomisés n'empêchait pas l'involution de la mamelle consécutive à l'opération. Lyons et Pencharz (1936) et de nombreux autres expérimentateurs, à leur suite, constatèrent que les œstrogènes étaient incapables de stimuler la mamelle atrophiée par hypophysectomie ou d'entretenir l'hypertrophie provoquée par les œstrogènes avant l'opération.

Les hormones stéroïdes sécrétées par l'ovaire sous l'influence de l'hypophyse ont encore besoin de l'action de l'hypophyse pour être actives sur le récepteur mammaire.

ACTION EXPERIMENTALE DES HORMONES STEROÏDES SUR LA CROISSANCE NORMALE ET PATHOLOGIQUE DE LA GLANDE MAMMAIRE

Croissance Normale

Hormones Stéroïdes de la Gonade

Dans l'édification du réseau mammaire, l'hormone œstrogène était considérée comme responsable de développer seulement les tubes galactophores. Cependant, chez différentes espèces d'animaux (souris, rats, cobayes, lapins et singes castrés), on a obtenu expérimentalement la formation d'acini par la seule administration d'une substance œstrogène.

L'hormone du corps jaune en synergie avec la folliculine était considérée comme chargée de former les acini. Mais, par l'administration de progestérone seule, au rat mâle adulte castré, nous avons provoqué la croissance des tubes et la

formation d'acini. La mamelle, par son extension et sa configuration est aussi comparable à celle de la femelle. Pour obtenir cette action mammogène, il faut cependant une administration journalière de 10–15 mg. d'hormone pendant environ 8 semaines. La souris se montre, dans nos expériences, comme étant beaucoup moins sensible à l'action mammogène de la progestérone. Par contre, chez le cobaye et le lapin castrés, nous n'avons pas obtenu une action stimulante de la mamelle par la seule administration de progestérone même à des doses importantes.

Les androgènes possèdent aussi une action stimulante sur la mamelle, ce qui expliquerait l'existence, chez certaines espèces animales, d'une mamelle relativement développée chez le mâle. Expérimentalement, nous avons constaté que seulement dans les espèces animales où la mamelle du mâle est assez développée, les androgènes (sans fonction œstrogène) ont une action stimulante. Chez la souris, le cobaye et le lapin mâles où la mamelle n'existe qu'à l'état de rudiments, le propionate de testostérone ne montre pas d'activité mammogène. Par contre, chez le rat mâle castré où la mamelle normalement est assez développée, le propionate des testostérone ou la méthyl-testostérone provoquent une stimulation mammaire. Cependant une différence apparait entre les rats mâles et femelles adultes, castrés. Chez les premiers, l'androgène agit sur les tubes sans provoquer la formation d'acini; chez les deuxièmes, le propionate de testostérone agit sur les tubes et développe les acini.

Bien que la progestérone et le propionate de testostérone stimulent la glande mammaire, leur action est fondamentalement différente. La progestérone féminise la mamelle. Agissant sur une mamelle de mâle atrophiée par castration, elle stimule les tubes, provoque l'apparition de bourgeons de croissance à l'extrêmité périphérique du réseau mammaire qui dépasse ses limites primitives, et développe des acini. Le propionate de testostérone ne féminise pas la mamelle: il stimule les tubes *pré-formés, provoque leur ouverture et* même leur élargissement, mais il est incapable de provoquer

la néo-formation de tubes par apparation de bourgeons de croissance ni la néo-formation d'acini mammaires; chez la femelle où les acini existent, il les développe. Il n'agit, en somme, que sur les parties de l'organe pré-existant à l'état d'ébauche, en provoquant leur hypertrophie et leur dilatation.

Hormones Stéroïdes Cortino-mimétiques

La désoxycorticostérone a été considérée, par différents auteurs, comme une substance capable de stimuler la glande mammaire, mais dans les expériences que nous avons réalisées chez la souris et le rat, ce pouvoir se montre très faible et inconstant malgré les fortes doses administrées. Parfois, chez les rats, un animal isolé présente une légère stimulation. Comme pour la progestérone et les androgènes, il existe une différence de sensibilité selon l'espèce animale envisagée. Chez le cobaye femelle castré, nous avons obtenu un développement mammaire par l'administration d'acétate de désoxycorticostérone.

La synergie qu'on obtient avec l'association d'un œstrogène et de la progestérone peut être aussi constatée en remplaçant cette dernière par l'acétate de désoxycorticostérone. Nous avons observé cette action chez le cobaye et le lapin castrés.

L'expérimentation a détruit la conception classique selon laquelle les œstrogènes provoquaient exclusivement la formation des tubes galactophores, et l'association d'œstrogène et de progestérone l'apparition d'acini. Cependant, pour obtenir, par une seule hormone le développement complet de la mamelle, il faut employer des quantités non physiologiques, parfois massives, de ces hormones. C'est avec l'association synergique de faibles doses d'œstrogène et de progestérone qu'on obtient le plus facilement le développement physiologique de la glande mammaire.

Antagonisme des Hormones Stéroïdes sur la Croissance Mammaire

Le fait que les substances œstrogènes et androgènes s'opposent dans leur action sur le vagin, a suggéré la recherche

d'un antagonisme des hormones stéroïdes sur le récepteur mammaire. Il faut cependant remarquer que cet antagonisme ne consiste pas dans une inhibition de l'action de l'œstrogène, mais dans une transformation de son action qui, d'œstrogène, se mue en progestative, l'action kératinisante se transformant en une action mucifiante. D'autre part, en raison du mécanisme spécial d'action des stéroïdes sur le récepteur mammaire, celui-ci ne peut être assimilé à d'autres récepteurs comme le vagin, la muqueuse utérine ou la prostate. Sur ceux-ci, *toute action des hormones stéroïdes agissant* seules ou associées (stimulation, synergie ou antagonisme) se réalise directement et peut être mise en évidence chez les animaux privés d'hypophyse. Par contre, pour toute action des hormones stéroïdes sur la mamelle, il faut le concours de l'hypophyse. Le problème de l'antagonisme des hormones sexuelles sur la glande mammaire doit être envisagé en tenant compte de cette nouvelle acquisition que toute action des hormones stéroïdes dépend, en définitive, de l'hypophyse.

Pour la glande mammaire, de nombreux essais expérimentaux et thérapeutiques ont été réalisés pour mettre à profit cette action antagoniste de l'hormone mâle et femelle: prophylaxie et traitement de l'adéno-carcinome mammaire spontané ou provoqué par l'œstrogène, chez la souris; traitement de l'hyperplasie kystique et du cancer mammaires chez la femme; et prophylaxie de récidives de ce dernier.

Pour tâcher d'éclaircir le mécanisme de l'action d'inhibition des androgènes, recherché sur la cellule mammaire cancérisée, il était intéressant de préciser s'il serait possible d'inhiber, par un androgène, la croissance normale de l'épithélium mammaire provoquée par un œstrogène. Pour ceci, de fortes doses d'une substance androgène ont été opposées à de faibles quantités d'œstrogène, suffisantes cependant pour provoquer un développement mammaire.* Nous avons choisi comme animaux d'expérience la souris, le cobaye et le lapin mâles, chez lesquels, au *préalable, nous avions constaté que de*

*Expériences inédites.

fortes doses d'androgène restaient sans action sur les ébau mammaires des animaux castrés.

Expériences chez la souris: Un groupe de souris mâles I a été traité avec 10 µg. de benzoate d'œstradiol, admin deux fois par semaine, par la voie sous-cutanée. Un deux groupe, en plus de l'œstrogène a reçu simultanément une 200 fois supérieure de méthyl-testostérone. Toutes mamelles des animaux ont été étudiées *in toto*, à trois, si huit semaines d'intervalle. Jusqu'à trois semaines, on const une inhibition partielle de la croissance, l'aire mamm est plus réduite et les bourgeons de croissance moins nc breaux, mais dans les délais de six et huit semaines, le trai ment associé s'est transformé en une action synergiq semblable à celle qui s'obtient avec la progestérone; l'a mammaire est plus étendue, les acini sont beaucoup pl nombreux, la dilatation des tubes est plus modérée.

Expériences chez le cobaye: Chez le cobaye, on administ soit 20 µg. de benzoate d'œstradiol, soit la même quanti d'œstrogène associé à une dose 200 *ou* 800 *fois* supérieure d propionate de testosterone, trois fois par semaine. Le mamelles sont étudiées aprés trois, six, neuf et douze semaine de traitement. Au terme de trois semaines, on constate comme chez la souris, une inhibition de la croissance de tubes qui sont aussi plus dégarnis. A partir de six semaines *on ne remarque plus de différences dans l'aire mammaire* des deux groupes et les mamelles, très hypertrophiées, présentent des tubes et des acini remplis de sécrétion.

Expériences chez le lapin: Chez le lapin, nous avons opposé à la dose d'œstrogène, une autre mille fois supérieure de propionate de testostérone: 5 µg. de benzoate d'œstradiol et la même dose associée à 5 mg. de propionate de testostérone, sont adminitrés, six fois par semaine. La deuxième paire de mamelles des animaux du groupe recevant seulement l'œstrogène et du groupe à traitement associé, a été étudiée *in toto* après deux, trois et quatre semaines de traitement. Au bout de deux semaines, les mamelles du deuxième groupe, l'aire étant similaire, présentent un réseau de tubes plus serrés et

moins dilatés. A la troisième semaine, on constate une aire mammaire inférieure dans la plupart des animaux, mais le réseau de tubes est toujours plus serré avec des bourgeons de croissance plus nombreux. A la quatrième semaine, l'aire mammaire est parfois inférieure, d'autres fois égale ou supérieure à celle des animaux traités par l'œstrogène seul. Chez les animaux où l'action synergique apparait, celle-ci se traduit ou par l'augmentation de l'aire mammaire, ou par la qualité de la réaction qui ressemble à celle qu'on obtient avec un traitement associé d'œstrogène et de progestérone ou par l'association de ces deux facteurs (Fig. 1).

L'action d'inhibition de croissance constatée à la troisième semaine, chez les trois espèces animales, se transforme en action synergique.

Avec ces mêmes doses d'œstrogène et de progestérone, nous n'avons pas pu mettre en évidence l'action antagoniste sur la mamelle du lapin qui a été observée par Lyons et MacGinty (Fig. 2).

L'expérimentation ne permet pas de conclure à un véritable antagonisme entre œstrogènes et androgènes. L'association de ceux ci fait apparaître que l'androgène agit à la façon de la progestérone.

Inhibition de la Croissance Mammaire par les Œstrogènes

Les substances œstrogènes administrées d'une façon réitérée, ont la propriété d'inhiber la sécrétion de certaines hormones hypophysaires: somatotrope, gonadotropes et thyréotrope. Etant donné que les œstrogènes agissent sur la glande mammaire avec la coopération d'un facteur hypophysaire, on conçoit la possibilité d'agir sur l'hypophyse pour essayer d'inhiber, par de fortes doses d'œstrogène, la sécrétion de cette substance hypophysaire mammogène. Gardner a signalé que 50 μg. d'œstrogène, par semaine, avaient un pouvoir inférieur pour stimuler la mamelle de la souris que des doses plus faibles. Nous avons confirmé cette observation chez les rats mâles castrés, chez lesquels 25 μg. par jour d'une

substance œstrogène provoquent un développement supérieur à celui obtenu avec 250 µg. par jour.

L'action thérapeutique de fortes doses d'œstrogène, que Haddow et ses collaborateurs ont observée sur le cancer mammaire de la femme, pourrait s'expliquer par cette action indirecte *par voie hypophysaire.*

Croissance Pathologique

Expérimentalement, l'administration réitérée d'une substance œstrogène aboutit à la formation d'une hyperplasie kystique de la mamelle. Cependant, toute hormone stéroïde, capable de stimuler la mamelle, ne produit pas cette action pathologique. Nous avons constaté que l'éthinyl-testostérone *ou pregneninolone, stéroïde synthétique, ayant des propriétés* œstrogène, progestative et androgène, est capable rapidement de féminiser la mamelle de la souris mâle, mais cependant, n'aboutit pas à provoquer la dégénérescence kystique. La progestérone, administrée d'une façon réitérée chez les rats où elle est active sur la mamelle, ne provoque pas non plus d'hyperplasie kystique. Les androgènes, par contre, administrés chez les rats mâles castrés, aboutissent, aprés un traitement *réitéré de plusieurs semaines à provoquer des* tubes dilatés d'aspect kystique.

Depuis que Goomaghtigh et Amerlinck (1930), par l'administration d'extrait de liquide folliculaire, ont produit des formations adénomateuses et l'hyperplasie kystique de la [illegible] femme. Mazer (1934), pensant à l'antagonisme hormonal, a suggéré de traiter cette maladie par les androgènes, et à la suite des résultats cliniques rapportés par Desmaret et Capitain (1937) *cette thérapeutique de la maladie de Reclus* est couramment employée.

Nous avons essayé de voir si cette thérapeutique pouvait être basée sur des données expérimentales. Pour cela, des rats femelles castrés ont reçu une quantité d'œstrogène

suffisante pour provoquer une hyperplasie kystique et à d'autres, nous avons associé à ce traitement le propionate de testostérone.* Les animaux ont reçu, soit 10 μg. de benzoate d'œstradiol par jour, pendant 4–6 semaines, soit la même dose d'œstrogène associée à une dose de propionate de testostérone 100 et 200 fois supérieure. L'étude des mamelles *in toto* et en coupes montre qu'au lieu d'empêcher l'apparition de l'hyperplasie kystique, le propionate de testostérone agit d'une façon synergique en provoquant des hypertrophies et hyperplasies plus importantes. Il semblerait donc que, si le propionate de testostérone agit d'une façon salutaire sur la mastopathie kystique, ce soit par un mécanisme autre que celui de l'antagonisme entre œstrogènes et androgènes.

RÔLE DE L'HYPOPHYSE DANS L'ACTION DES HORMONES STEROÏDES SUR LA GLANDE MAMMAIRE

Action des Hormones Stéroïdes Seules ou Associées en Absence de l'Hypophyse

Depuis la première constatation négative de Selye, Collip et Thomson, de nombreuses recherches ont été réalisées pour tâcher de stimuler la glande mammaire des animaux hypophysoprivés, par l'administration isolée ou associée d'hormones stéroïdes. La plupart des auteurs, dans des expériences réalisées chez la souris, le rat et le cobaye, ont constaté des résultats négatifs. Cependant, dans ces dernières années des travaux ont encore attribué aux œstrogènes ou aux androgènes une action stimulante sur la mamelle du rat hypophysectomisé. Nous avons réalisé des expériences successives afin d'étudier l'action que les hormones stéroïdes seules ou associées, l'extrait d'ovaire et la sécrétion du propre ovaire de l'animal pourraient avoir sur la mamelle du rat hypophysectomisé.

*Expérience inédite.

Nous avons signalé qu'une association d'œstrogène et d'acétate de désoxycorticostérone provoque une stimulation mammaire chez le rat mâle hypophysectomisé. Cette expérience a été confirmée par Gardner chez la souris hypophysectomisée. Mais cette stimulation est relativement faible et il faut la ramener à sa vraie place. Parmi les nombreuses expériences que nous avons réalisées, nous en avons retenu certaines qui, à cause du long traitement et des hautes doses administrées, permettront de se rendre compte de l'importance toute relative de cette stimulation. Les détails sont consignés dans le tableau ci-joint Table I.

De l'analyse de ces cas, il résulte qu'une stimulation peut être obtenue chez le rat hypophysectomisé par l'administration d'un œstrogène naturel ou par l'association de cet œstrogène et de progestérone ou d'acétate de désoxycorticostérone. Mais, cette stimulation reste limitée au territoire des gros tubes collecteurs aboutissant au mamelon et est très faible, étant données et la durée du traitement, et les doses massives administrées. Or, avec des doses très inférieures, chez l'animal castré, on provoque d'énormes hyperplasies *et même des phénomènes pathologiques*. Il semble que l'épithélium mammaire des gros tubes collecteurs aboutissant au mamelon, soit un épithélium de transition jouissant, en un certain degré, de la propriété de réagir comme le *mamelon réagit chez les animaux sans hypophyse*. Nous n'avons, par contre, jamais constaté de stimulation du réseau mammaire secondaire situé au delà de ces gros tubes collecteurs. Avec de très fortes doses d'un œstrogène artificiel, *nous n'avons même pas observé cette action stimulante* limitée à la zone proche du mamelon. Il semble qu' une différence se manifeste entre œstrogènes naturels et artificiels dans ce cas particulier.

Action des Extraits Ovariens en Absence de l'Hypophyse

Il nous a semblé intéressant de vérifier si un extrait d'ovaire n'avait pas, pour stimuler la mamelle, de capacités différentes

ACTION D'UNE SUBSTANCE ŒSTROGÈNE SEULE OU ASSOCIÉE A LA PROGESTÉRONE OU A L'ACÉTATE DE DÉSOXYCORTICOSTÉRONE SUR LA MAMELLE DU RAT HYPOPHYSECTOMISÉ

Sexe	Survie de l'animal après hypophysectomie (en jours)	Durée du traitement (en jours)	Voie d'administration	Dose totale d'hormone stéroïde administrée en mg. Œstrogène	Progestérone	Acétate de désoxycorticostérone	Réaction mammaire	Poids initial en g.	Poids final en g.	Température C° rectale profonde le jour du sacrifice
femelle castrée	87	0	-	0	0	0	Atrophie	151	125	35°9
femelle castrée	87	60	comprimé de 10,6 mg inséré sous la peau	D.S.B.(1) 8,5			Atrophie	143	98	35°8
mâle	81	0	—	0	0	0	Atrophie	158	132	36°8
mâle	81	50	voie orale	D.S.B. 11,45	0	0	Atrophie	174	105	32°8
femelle castrée	98	0	—	0	0	0	Atrophie	113	85	36°
femelle castrée	98	92	comprimé de 8 mg inséré sous la peau	B.E.(2) 1,6	0	0	+	140	93	non prise
mâle	70	0	—	0	0	0	Atrophie	148	108	36°5
mâle	70	56	sous-cutanée	B.O.(3) 1,6	51	0	Atrophie	148	103	35°
mâle	98	0	—	0	0	0	Atrophie	120	95	34°8
mâle	98	84	sous-cutanée	B.O. 2,4	75	0	+	151	95	31°2
femelle	84	0	—	0	0	0	Atrophie	64	66	35°5
femelle	84	50	sous-cutanée	B.O. 5,1	0	8	+	50	55	31°
mâle	98	0	—	0	0	0	Atrophie	121	124	36°7
mâle	98	53	sous-cutanée	B.O. 2,2	0	11,5	+	138	92	non prise

(1) D.S.B. = Diéthyl Stilbestrol, (2) B.E. = Benzoate d'œstrone, (3) B.O. = Benzoate d'œstradiol.
(4) Légère stimulation.

Nous avons signalé qu'une association d'œstrogène et d'acétate de désoxycorticostérone provoque une stimulation mammaire chez le rat mâle hypophysectomisé. Cette expérience a été confirmée par Gardner chez la souris hypophysectomisée. Mais cette stimulation est relativement faible et il faut la ramener à sa vraie place. Parmi les nombreuses expériences que nous avons réalisées, nous en avons retenu certaines qui, à cause du long traitement et des hautes doses administrées, permettront de se rendre compte de l'importance toute relative de cette stimulation. Les détails sont consignés dans le tableau ci-joint Table I.

De l'analyse de ces cas, il résulte qu'une stimulation peut *être obtenue chez le rat hypophysectomisé par l'administration* d'un œstrogène naturel ou par l'association de cet œstrogène et de progestérone ou d'acétate de désoxycorticostérone. Mais, cette stimulation reste limitée au territoire des gros tubes collecteurs aboutissant au mamelon et est très faible, étant données et la durée du traitement, et les doses massives administrées. Or, avec des doses très inférieures, chez l'animal castré, on provoque d'énormes hyperplasies et même des phénomènes pathologiques. Il semble que l'épithélium mammaire des gros tubes collecteurs aboutissant au mamelon, soit un épithélium de transition jouissant, en un certain degré, de la propriété de réagir comme le mamelon réagit chez les animaux sans hypophyse. Nous n'avons, par contre, jamais constaté de stimulation du réseau mammaire secondaire situé au delà de ces gros tubes collecteurs. Avec de très fortes doses d'un œstrogène artificiel, nous n'avons même pas observé cette action stimulante limitée à la zone proche du mamelon. Il semble qu' une différence se manifeste entre œstrogènes naturels et artificiels dans ce cas particulier.

Action des Extraits Ovariens en Absence de

Table I

ACTION D'UNE SUBSTANCE ŒSTROGÈNE SEULE OU ASSOCIÉE A LA PROGESTÉRONE OU A L'ACÉTATE DE DÉSOXYCORTICOSTÉRONE SUR LA MAMELLE DU RAT HYPOPHYSECTOMISÉ

Sexe	Survie de l'animal après hypophysectomie (en jours)	Durée du traitement (en jours)	Voie d'administration	Dose totale d'hormone stéroïde administrée en mg.			Réaction *mammaire*	Poids initial en g	Poids final *en g*	Température C° rectale profonde *le jour du* sacrifice
				Œstrogène	Progestérone	*Acétate de désoxycorticostérone*				
femelle castrée	87	0	—	0	0	0	Atrophie	151	125	35°0
femelle castrée	87	80	comprimé de 10,6 mg inséré sous la peau	D S.B.(1) 8,5			Atrophie	143	98	35°8
mâle	81	0	—	0	0	0	Atrophie	158	132	36°8
mâle	81	56	voie orale	D.S.B. 11,45	0	0	Atrophie	174	105	32°8
femelle castrée	98	0	—	0	0	0	Atrophie	113	85	36°
femelle castrée	98	92	comprimé de 8 mg inséré sous la peau	B.E (2) 1,6	0	0	±	140	93	non prise
mâle	70	0	—	0	0	0	Atrophie	148	108	36°5
mâle	70	56	*sous-cutanée*	B.O.(3) 1,6	51	0	Atrophie	148	103	35°
mâle	98	0	—	0	0	0	Atrophie	120	93	34°8
mâle	98	84	sous-cutanée	B.O 2,4	75	0	±	151	95	31°2
femelle	84	0	—	0	0	0	Atrophie	64	66	35°5
femelle	84	56	sous-cutanée	B.O. 3,1	0	8	±	59	55	31°
mâle	98	0	—	0	0	0	Atrophie	121	124	36°7
mâle	98	56	sous-cutanée	B O. 2,2	0	11·5	±	138	92	non prise

(1) D.S B. = Diéthyl-Stilbœstrol, (2) B E. = Benzoate d'œstrone, (3) B O = Benzoate d'œstradiol.
(±) Légère stimulation.

à celles des hormones stéroïdes cristallisées. Cette expérience, qui a été réalisée en collaboration avec Girard et Sandulesco, est restée à l'état de premier résultat,* et une étude approfondie avec des extraits concentrés et fractionnés n'a pas été réalisée. Toutefois, un extrait brut d'ovaire de truie a été administré, chez le rat mâle hypophysectomisé, à mamelles atrophiées, et nous avons constaté une faible stimulation mammaire qui, à l'inverse de celle qui a été obtenue auparavant avec les hormones synthétiques, se localise dans les tubes du réseau périphérique. Etant donné qu'il s'agit d'un extrait brut, la dose des substances actives est certainement faible, et sans aucun rapport avec les quantités très importantes d'hormones stéroïdes cristallisées que nous avons administrées dans l'expérience précédente.

Action des Hormones Stéroïdes Secretées par l'Ovaire, sous l'Influence de la Gonadotrophine Sérique de Jument Gravide, sur la Mamelle des Animaux sans Hypophyse

Les substances œstrogènes, même à des doses peu élevées, administrées journellement, provoquent l'amaigrissement des animaux normaux. Mais leur action toxique devient considérable si elles sont administrées à des animaux sans hypophyse. Ces animaux supportent mal le traitement et même quand on réussit à les maintenir en équilibre, il en résulte une chute de poids plus considérable que chez les témoins, une perte plus importante de l'appétit et une baisse plus accentuée de la température rectale profonde. Cette baisse de température qui, chez les animaux hypophysectomisés témoins atteint 2–3 degrés, se chiffre de 5–7 degrés chez les animaux traités par des substances œstrogènes. Dans les expériences réalisées avec des extraits bruts d'ovaire, la solution huileuse, administrée en quantités importantes, se résorbe mal, bien que les animaux se maintiennent dans un état général plus satisfaisant.

**Expérience inédite.*

Nous avons constaté par contre que l'administration de gonadotrophine, chez les animaux sans hypophyse, n'était suivie ni d'amaigrissement, ni de chute de température plus considérable. Nous avons donc envisagé de stimuler l'ovaire par une gonadotrophine, pour voir la répercussion que ce traitement aurait sur la mamelle des animaux sans hypophyse. Nous avons choisi la gonadotrophine du serum de jument gravide, active sur les ovaires des animaux sans hypophyse, et d'origine non hypophysaire, puisque sécrétée par le placenta. D'autre part, la propre sécrétion de l'ovaire de l'animal allait agir sur la mamelle. Pour ceci, de jeunes rats femelles, vierges, d'un poids de 100 à 150 g. ont été hypophysectomisés. Environ trois jours aprés l'opération, avant que l'ovaire et la mamelle n'entrent en régression, on commence le traitement par voie sous-cutanée ou intra-péritonéale. matin et soir, à la dose journalière de 20 à 30 unités-rat, pendant une période de 3–4 semaines. Les ovaires des animaux traités atteignent des poids considérables de 200 à 483 mg. Ils sont remplis de gros follicules parfois hémorragiques et de nombreux corps jaunes. Les ovaires des témoins pèsent de 10 à 15 mg. L'étude comparative, *in toto* et en coupes, des mamelles de ces deux groupes d'animaux, montre, que chez les animaux traités, l'involution mammaire est empêchée (Fig. 3). Si un tel traitement est poursuivi pendant plusieurs semaines, l'ovaire devient réfractaire à la stimulation, entre en involution et la mamelle régresse.

Ce résultat pourrait être attribué à une action synergique de la gonadotrophine et de la sécrétion ovarienne. Or, chez les rats castrés et hypophysectomisés, l'administration de gonadotrophine associée à un stéroide œstrogène et progestatif n'a pas empêche l'involution mammaire. Il faudrait interpréter cette constatation en admettant, ou bien que la sécrétion du propre ovaire de l'animal se montre plus actif sur la mamelle, ou bien que le bon état général de celui-ci favorise la réaction mammaire.

Action, sur la Glande Mammaire, des Implantats d'Hypophyses

La constatation que les substances œstrogènes sont inactives ... Turner et ses collabora- ... 'existence d'hormones ... et qui stimuleraient *directement* la mamelle. Une hormone mammogène serait sécrétée sous l'influence de la folliculine et provoquerait la croissance des tubes, une deuxième serait sécrétée sous l'influence de la progestérone et produirait la prolifération d'*acini*, la folliculine et la progestérone n'ayant d'autre rôle que d'exciter l'hypophyse. Cette hypothèse prenait assise sur la constatation expérimentale que les hypophyses des animaux soumis à un traitement œstrogène et celles des animaux gravides étaient seules capables de stimuler la mamelle des animaux castrés-hypophysectomisés.

Nous avons réalisé des expériences pour déceler l'existence de ces hormones hypothétiques.

A des rats mâles hypophysectomisés, à mamelles atrophiées, nous avons fait des implantations répétées de trois sortes d'hypophyses provenant de rats normaux, de rats castrés ou de rats ayant subi au préalable un traitement œstrogène pendant trois mois. Les deux premières sortes d'hypophyses provoquent une stimulation mammaire. Par contre, les hypophyses de la troisième catégorie ne produisent qu'une très faible stimulation. Dans les deux premiers cas, les hypophyses implantées ont stimulé les testicules, dans le troisième, les hypophyses, pauvres en hormones gonadotropes, les ont à peine stimulé. Si la mamelle, dans ce dernier cas, n'a pas été stimulée, il semble que ce soit à cause du manque de sécrétion d'hormones stéroïdes par la gonade, d'où manque d'un des éléments nécessaires pour l'action synergique. Pour vérifier cette interprétation, chez les rats castrés-hypophysectomisés, des hypophyses provenant d'animaux œstrogénisés sont implantées en leur associant l'administration d'une hormone stéroïde mammogène. Dans ce cas, on



A



B

FIG 3 A Mamelle atrophiée de jeune rat femelle vierge, 6 semaines après l'hypophysectomie B idem, après administration pendant les 5 dernières semaines de gonadotrophine sérique

Action, sur la Glande Mammaire, des Implantats d'Hypophyses

La constatation que les substances œstrogènes sont inactives en l'absence de l'*hypophyse, a conduit Turner et ses collaborateurs*, à émettre l'hypothèse de l'existence d'hormones spécifiques sécrétées par l'hypophyse et qui stimuleraient directement la mamelle. Une hormone mammogène serait sécrétée sous l'influence de la folliculine et provoquerait la croissance des tubes, une deuxième serait sécrétée sous l'influence de la progestérone et produirait la prolifération d'acini, la folliculine et la progestérone n'ayant d'autre rôle que *d'exciter l'hypophyse. Cette hypothèse prenait* assise sur la constatation expérimentale que les hypophyses des animaux soumis à un traitement œstrogène et celles des animaux gravides étaient seules capables de stimuler la mamelle des animaux castrés-hypophysectomisés.

Nous avons réalisé des expériences pour déceler l'existence de ces hormones hypothétiques.

A des rats mâles hypophysectomisés, à mamelles atrophiées, nous avons fait des implantations répétées de trois sortes d'hypophyses provenant de rats normaux, de rats castrés ou de rats ayant subi au préalable un traitement œstrogène pendant trois mois. Les deux premières sortes d'hypophyses provoquent une stimulation mammaire. Par contre, les *hypophyses de la troisième catégorie ne produisent* qu'une très faible stimulation. Dans les deux premiers cas, les hypophyses implantées ont stimulé les testicules, dans le troisième, les hypophyses, pauvres en hormones gonadotropes, les ont à peine stimulé. Si la mamelle, dans ce dernier cas, n'a pas été stimulée, il semble que ce soit à cause du manque de sécrétion d'hormones stéroïdes par la gonade, d'où manque d'un des éléments nécessaires pour l'action synergique. Pour vérifier cette interprétation, chez les rats castrés-hypophysectomisés, des hypophyses provenant d'animaux œstrogénisés sont implantées en leur associant l'administration d'une hormone stéroïde mammogène. Dans ce cas, on



A



B



C



D

Fig 5 A Mamelle de rat femelle adulte normal Témoin
B Ide
Stimul
traiten
nombr
dilatés



A



B



C

FIG. 4. A Mamelle de jeune rat mâle castré, 110 hours après

[illegible]

bourgeons de croissance. ×6,5

constate une très nette stimulation mammaire (Fig. 4). Cette expérience montre que l'hormone stéroïde est nécessaire pour obtenir une stimulation mammaire plus importante.

Cependant les hypophyses des rats normaux possèdent la capacité de stimuler la mamelle du rat hypophysectomisé et castré. Mais dans ce cas, la stimulation est faible et se caractérise par l'apparition de petits bourgeons de croissance. L'action devient plus intense si, en plus des hypophyses, on administre une hormone stéroïde mammogène. L'intervention dans la réaction d'autres glandes endocrines n'est pas à écarter puisque si, à des rats castrés, à mamelle atrophiée, on extirpe la thyroïde et la surrénale, et on réalise des implantations d'hypophyses, la stimulation mammaire est nulle ou très faible. D'autre part Lacassagne et Dubois n'ont pas observé un pouvoir particulier des hypophyses des souris gravides pour stimuler la mamelle de la souris castrée.

Si l'hypophyse secrète une hormone spécifique pour stimuler directement la glande mammaire, cette hormone doit se trouver dans le sang des animaux à glandes mammaires fortement développées. Nous avons essayé de la déceler dans le serum sanguin des animaux soumis à un traitement réitéré par les œstrogènes et dans le serum de jument gravide et de femme enceinte. Ces produits administrés à des rats castrés-hypophysectomisés n'ont pas donné de stimulation mammaire.

Ces expériences montrent que l'hypophyse et les hormones [illegible]

aussi contredite par les constatations de Mussio-Fournier, Albrieux et Buño (1937) et Lyons et Sako (1940) sur l'activité des œstrogènes en application locale.

Synergie des fractions hypophysaires et des hormones stéroïdes mammogènes. La synergie des différentes fractions hypophysaires avec les œstrogènes a été recherchée en utilisant le rat castré-hypophysectomisé. Nous avons essayé les fractions gonadotrope, thyréotrope et la prolactine, et seulement cette dernière associée à l'œstrogène stimulait la mamelle. Ce



A



B

Fig 6 A Mamelle de rat femelle adulte Témoin B Idem, après administration de propyl-thiouracile (un mg par jour) pendant 6 semaines Dilatation des tubes qui contiennent une sécrétion, et prolifération d'acini. ×44

d'hypothyroïdisme crée un terrain favorable à une action intensifiée des hormones stéroïdes qui conduit à l'apparition des lésions pathologiques mammaires.

Dans une série d'expériences, nous avons mis en évidence le rôle de la thyroïde dans le déclanchement des phénomènes pathologiques mammaires par les hormones stéroïdes.

(i) L'administration à des rats castrés-thyroïdectomisés d'un stéroïde mammogène provoque une action plus intense que chez les rats seulement castrés.

(ii) Des rats femelles adultes sont thyroïdectomisés et traités par une gonadotrophine à la dose de 10 u.i. par jour, pendant quelques jours. Tandis que chez les animaux normaux traités, on constate une légère stimulation mammaire, chez les thyroïdectomisés, les mamelles sont le siège d'une hypertrophie et d'une hyperplasie qui prennent parfois un caractère kystique (Fig. 5).

(iii) Nous avons ensuite étudié la répercussion mammaire de la production chez les rats femelles adultes, âgés d'environ 14 mois, d'un état d'hypothyroïdisme léger. Les animaux ont été soumis à l'administration sous-cutanée de faibles doses de propyl-thiouracile et les mamelles étudiées *in toto* et en coupes à des délais variant de 4 à 10 semaines. Nous avons constaté dans les premières semaines une prolifération d'acini et ensuite l'apparition de phénomènes d'hyperplasie kystique. Parfois, il s'agit seulement d'une extrême dilatation des tubes; d'autres fois, de prolifération de grappes d'acini devenus kystiques, parsemés dans la glande; d'autres fois, d'apparition de grands kystes isolés (Fig. 6). Ces manifestations rapellent les différents degrés de la mastopathie kystique chez la femme.

(iv) Dans une autre expérience, nous avons étudié quel serait l'effet sur la glande mammaire, de l'ablation de la thyroïde* chez le rat femelle adulte, âgé de 14 à 18 mois. *L'examen des mamelles est réalisé à des délais* de 4 à 10

*Pendant les 10 jours précédant la thyroïdectomie, les animaux reçoivent 1 mg de propyl-thiouracile par jour, par la voie sous-cutanée ou intrapéritonéale, ce qui réduit pratiquement à zéro la mortalité consécutive à l'opération.

résultat a été aussi rapporté par différents auteurs avec les fractions de croissance et corticotrope.

Toutefois, ces expériences ont seulement une valeur d'orientation et l'existence d'une hormone mammogène ne pourra être prouvée que lorsqu'on disposera d'hormones hypophysaires à l'état pur.

Mécanisme de l'Action Stimulante des Hormones Stéroïdes sur la Glande Mammaire

Le mécanisme d'action des hormones stéroïdes sur la croissance normale de la glande mammaire se présente, à l'heure actuelle, comme un problème complexe très loin de la simplicité de la conception première d'un *déterminisme ovarien*. Les substances œstrogènes ne sont actives sur la glande mammaire qu'avec la coopération hypophysaire. Mais le mécanisme de cette coopération n'est pas éclairci. Cette action associée peut s'expliquer par:

(*a*) une action préparatoire ou mordançage, exercée par l'hypophyse sur l'épithélium mammaire.

(*b*) une synergie entre une substance hypophysaire et l'hormone stéroïde.

(*c*) une transformation de la constitution chimique de la substance stéroide produite sous l'influence de l'hypophyse et rendant celle-là active sur l'épithélium mammaire.

Le métabolisme de l'hormone stéroïde serait provoqué *in situ* par des enzymes contrôlés par l'hypophyse. Chez les animaux hypophysectomisés, ces enzymes disparaitraient ou deviendraient inactifs, d'où manque d'activité de l'œstrogène introduit dans la circulation générale ou appliqué *in situ*. Cette hypothèse permettrait un accord entre le rôle joué par l'hypophyse et l'action locale exercée par les œstrogènes.

INTERVENTION DE LA THYROÏDE DANS L'ACTION DES HORMONES STEROÏDES SUR LA GLANDE MAMMAIRE

Dans la coopération de l'ovaire et de l'hypophyse pour agir sur la croissance mammaire, la thyroïde intervient. Un état



A



B



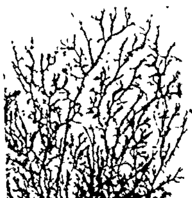
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semaines après l'opération et nous avons constaté, chez la plupart des animaux, une dilatation des tubes, une prolifération d'acini, des formation adénomateuses et l'apparition d'acini kystiques, soit en grappes isolées, soit généralisés à toute la glande. L'administration de thyroxine empêche la réaction mammaire (Fig. 7).

(v) Chez le rat femelle castré, l'administration d'une quantité de benzoate d'œstradiol de 2·5 μg. trois fois par semaine, capable, à peine, d'empêcher l'involution de la mamelle, produit chez le rat castré-thyroïdectomisé, la dilatation des tubes et une abondante prolifération d'acini en grande partie kystiques. Si les rats castrés sont soumis à un traitement par le benzoate d'œstradiol à une dose de 5 μg., trois fois par semaine, on obtient, au bout de six semaines de traitement, des manifestations très modérées d'hyperplasie kystiques; si à ces mêmes animaux, on ajoute un traitement par la thyroxine, on empêche l'apparition de toute stimulation normale ou pathologique (Fig. 8).

(vi) Dans cette expérience, nous avons étudié l'action que le propionate de testostérone pourrait avoir sur la mamelle dans un état d'hypothyroïdisme (expérience inédite). Des rats femelles adultes âgés de 14 à 18 mois sont castrés et traités, soit par le propionate de testostérone, soit par une association de propyl-thiouracile et de propionate de testostérone, soit thyroïdectomisés et soumis au traitement par le propionate de testostérone. Le propyl-thiouracile et l'androgène ont été administrés aux doses journalières d'un ou deux milligrammes.

Les femelles castrées traitées par le propionate de testostérone, présentent une stimulation mammaire qui dépasse l'état normal, avec élargissement des tubes. Chez les animaux des deux autres groupes, propyl-thiouracile ou thyroïdectomie associés à l'androgène, la mamelle est hypertrophiée et hyperplasiée avec signes de dégénérescence kystique (Fig. 9). Microscopiquement, on observe une dilatation des tubes et des acini qui contiennent une sécrétion; des proliférations épithéliales de type papilliforme et une desquamation épithéliale



A



B



C



D

Fig 9 A Mamelle de rat femelle adulte, castré depuis 6 semaines, atrophie B Idem, castré, traite par le propionate de



A



B



C



D

FIG 8 A. Mamelle de rat femelle adulte castré, après administration de 2 5 µg de benzoate d'œstradiol, 3 fois par semaine, pendant 6 semaines B Idem, castré et thyroïdectomisé et recevant le même traitement que le precedent, hyperplasie kystique

abondante contenant des cellules spongieuses à l'intérieur des tubes et des acini, ainsi qu'une réaction conjonctive périglandulaire, parfois intense, toutes ces lésions étant semblables à celles qu'on observe dans la maladie kystique, chez la femme (Fig. 10). La muqueuse utérine de ces animaux présente aussi une hyperplasie kystique.

D'après ces expériences, dans les états d'hypothyroïdisme, les androgènes peuvent agir d'une façon pathologique sur la glande mammaire. Il faudrait rapprocher ces résultats du fait que, dans la clinique, la mastopathie et le cancer mammaire apparaissent le plus souvent à un âge où la fonction thyroïdienne est à son déclin et où la sécrétion androgène de l'ovaire augmente.

Le mécanisme de production expérimentale de l'hyperplasie kystique par les œstrogènes serait le suivant: les substances œstrogènes agissent sur l'hypophyse en y provoquant une diminution et, à la longue, une suspension de la sécrétion d'hormone thyréotrope, d'où un état d'hypothyroïdisme qui permet à l'œstrogène de produire des lésions pathologiques sur la glande mammaire. Dans le cas d'hypothyroïdisme expérimental, ce processus s'abrège. L'hypothyroïdisme spontané, en clinique, serait un terrain favorable à l'éclosion de la mastopathie.

Cet ensemble de résultats montre que la déficience de la thyroïde joue un rôle important dans la production, par les hormones stéroïdes, des lésions pathologiques de la mamelle.

RÉSUMÉ

1. Les hormones stéroïdes capables de stimuler la glande mammaire, montrent des différences dans leur action qui

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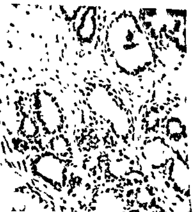
Fig. 10. Différents aspects microscopiques qu'on observe dans les mamelles

épidermoïde; cellules à protoplasme clair et desquamation épithéliale abondante à l'intérieur d'un tube galactophore. ×200.

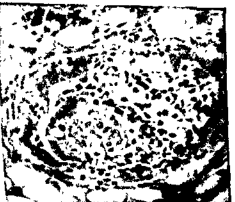

A


B


C







un état d'hypothyroïdisme ou inhibée par administration de thyroxine.

Dans un état d'hypothyroïdisme expérimental, les androgènes déclenchent rapidement une hyperplasie kystique de la mamelle.

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dépend de leur constitution chimique et de l'espèce animale chez laquelle elles sont administrées. Les œstrogènes et la progestérone provoquent la croissance des tubes et la formation d'acini mammaires, mais l'activité de la progestérone est très faible. Le propionate de testostérone stimule les tubes et les acini pré-existants, mais ne produit ni de bourgeons de croissance, ni d'acini. La synergie qu'on obtient par l'administration simultanée d'œstrogène et de progestérone peut être aussi obtenue par d'autres hormones stéroïdes ayant une action progestative, comme l'acétate de désoxycorticostérone et la testostérone. Le développement le plus rapide et complet de la glande mammaire est obtenu par l'action synergique de faibles doses d'œstrogène et de progestérone.

Les hormones stéroïdes sécrétées par la surrénale ne jouent pas un rôle appréciable dans la croissance normale de la glande mammaire.

Il n'est pas possible, expérimentalement, de mettre en évidence un véritable antagonisme entre œstrogènes et androgènes, sur la mamelle. L'administration associée de ces hormones stéroïdes donne une action synergique.

2. Les hormones stéroïdes n'agissent sur la glande mammaire qu'avec la coopération de l'hypophyse. Il s'agit d'une synergie sur le récepteur. Mais le mécanisme intime de cette action n'est pas encore éclairci. Bien que l'implantation d'hypophyses produise, chez les animaux castrés et hypophysectomisés, une certaine stimulation mammaire, cette action est très renforcée si on associe un stéroïde mammogène. La coopération des hormones stéroïdes et de l'hypophyse est nécessaire pour obtenir un développement complet de la glande mammaire.

3. La thyroïde joue un rôle de protection contre l'action pathologique de certaines hormones stéroïdes. Un état d'hypothyroïdisme expérimental favorise l'apparition d'hyperplasie kystique de la mamelle.

L'hyperplasie kystique de la mamelle, provoquée par l'administration de substances œstrogènes, peut être favorisée par

in the thyroidectomized animal could be produced with a fraction of the minimal effective dose of estrogen in an intact animal, and that

whatsoever.

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DISCUSSION

PULLINGER: Do you find this cystic disease in rats spontaneously? In mice, although you can produce the cystic disease with non-physiological large doses of œstrogen, I never saw it occurring spontaneously in the R-III strain. I have seen it in the Simpson strain. Does this condition develop in mice which are simply thyroidectomized and not treated, as opposed to rats?

CHAMORRO: We have not done these experiments on mice.

GARDNER: I am amazed to find that the testosterone when given with œstrogen has augmented the growth of the mammary gland as much as Dr. Chamorro has described. In our long-term experiments we have accumulated considerable evidence that the mammary growth was impaired by the addition of testosterone in the ratio of 1:1000. We have not, however, used animals of the R-III strain. This has been done primarily using animals of the C_3H strain.

FOLLEY: Do you believe that the effect of thyroidectomy on mammary development in non-spayed females is due to an effect on the metabolism of endogenous œstrogen? Does it mean that in thyroidectomized animals the œstrogen is not inactivated so rapidly as it is in an intact animal?

in the thyroidectomized animal could be produced with a fraction of the minimal effective dose of œstrogen in an intact animal, and that thyroidectomy does increase the sensitivity, to a given dose of œstrogen in the rat. In clinical observations we have attempted to see whether in people suffering from [illegible] there was any indication of thyroid [illegible]

whatsoever.

THE EFFECT OF STEROIDS ON THE INCIDENCE OF MAMMARY TUMOURS IN MICE

O. MÜHLBOCK

I AM glad to have the opportunity to discuss with you some experiences we at Amsterdam have had in investigating the effect of certain steroid hormones on the development of mammary cancer in mice. Our experiences do not include all steroid hormones but are mainly concerned with the influence of sex hormones. Before giving the results, I should like to say a few words on the technique of administration. All experiments were made with hormones in the form of pellets of 1–2 mg., administered subcutaneously. Pellets of pure progesterone and pure testosterone propionate were given. Preliminary tests showed that the application of pure œstrone produced too high a mortality; 25 per cent œstrone mixed with 75 per cent cholesterol is tolerated and effective. In order to clarify the results of our experiments, a survey of the strains and hybrids used in the experiments, and the abbreviations of their names, is given in Table I.

Table I

STRAINS AND HYBRIDS EXAMINED

d = Dilute-brown-(Little)	high-cancer-strain
B = C_{57} Black-(Little)	low-cancer-strain
O = O20-Leeuwenhoekhuis	low-cancer-strain
A = A Strong	cancer-strain
dB = F_1 (♀ dilute brown × ♂ C_{57} Black)	
dO = F_1 (♀ dilute brown × ♂ O20)	

In the first experiments normal females of the d-strain were treated with hormone pellets (Table II). Table II and the next ones are arranged in the following manner: "No." represents the number of animals at the beginning of the

experiment; "No. living at tumour age" includes only those that survived to tumour age, as determined by the age of the first animal in the particular group to develop a tumour. For your convenience the mammary-tumour-incidence is always given as a percentage. "Tumour age" in the next column means the age when tumours were first palpable. "Non-tumour age" means the age at death of non-tumorous mice.

Table II

FEMALES OF THE D-STRAIN TREATED WITH HORMONE PELLETS

	No	*No. living at tumour age*	*Percentage mammary tumour incidence*	*Tumour age Months*	*Non-tumour age Months*
Virgins untreated		87	59	18	18
Breeders		122	59	15	19
Œstrone at one month of age	35	26	62	14	15
Œstrone first day of life	49	29	45	14	16
Progesterone first day of life	17	15	53	13	14
Testosterone first day of life	24	8	25	18	18

The first group of untreated females acted as controls. The tumour percentage was the same in breeders and virgins; the average tumour age in breeders, however, was three months earlier. Œstrone treatment at the age of one month showed the same percentage of tumour incidence, the tumour age corresponding with that of breeders. Treatment at the first day of life gives the same result. There is no significant difference in the incidence between the two treated groups. The mortality in the treated groups was very high. Treatment with pure progesterone pellets on the first day showed the same effect as treatment with œstrone. As could be expected, testosterone given as pure testosterone propionate

pellets had an inhibiting effect, but the mortality was so high that no safe conclusions could be drawn.

The next experiments were made on males (Table III).

Table III

	No.	*No. living at tumour age*	*Percentage mammary tumour incidence*	*Tumour age Months*	*Non-tumour age Months*
♂d	78	44	4	14	17
♂d castrated .	71	64	14	12	14
♀d virgins untreated . .		87	59	18	18
♂dB . . .	20	19	21	15	21
♂dB castrated . . .	8	8	75	15	20
♀dB virgins untreated		80	67	19	21
♂dO	27	20	40	10	16
♂dO castrated . .	21	21	71	12	24
♀dO virgins untreated		73	93	14	16
♂A castrated .	27	26	46	10	10

Table III shows the results in normal and castrated males treated with œstrone pellets at the age of one month as compared with the incidence of mammary tumours in virgin females. Three groups were investigated: d, dB and dO. The incidence of cancer in normal males is considerably lower than in untreated virgin females. This is especially striking in the normal males of the Dilute-brown strain, in which only a few mammary tumours occur. The mortality of the treated d-males is rather high. Only 44 of the 78 d-males reached the tumour-age. The average tumour-age in the treated males is earlier than in the virgins.

The first question which arises when determining the cause of these low figures in normal males is whether the mammary gland has been developed sufficiently as a result of the treatment. Therefore in treated normal d-males, whole mount mammary preparations were made of 34 animals after varying periods of treatment. In only 9 per cent could the mammary gland be considered to be satisfactorily developed.

An antagonistic action of the testes is first considered when seeking the cause of the absence of growth of the mammary gland in these males. Gardner found an antagonism between œstrone and testosterone in respect of the development of the mammary gland. We showed this antagonism in the following manner: we used the method of Turner and Lewis to determine the threshold value at which œstrone evokes the first signs of growth in the mammary gland; these first signs are formations of club-shaped swellings at the ends of the ducts; the swellings are accumulations of cells, among which many mitoses are to be found (*Acta Brevia Neerlandica*, **1948**, **16**, **1**).

Comparison of the threshold values of normal and castrated males shows a considerable difference, especially in males of the d-strain: 1 μg. œstrone in castrated against 50 μg. in non-castrated animals. No such large difference was observed in the two other strains (B and O20) investigated. With the same method it could be shown that testosterone is capable of counteracting the influence of œstrone on the mammary glands.

The next experiment was therefore carried out on castrated males (Table III). In the d-strain the frequency after castration remains low; lower than in castrated males of the A-strain. Experiments on non-castrated males of the A-strain have not yet been completed. However, Bonser has recently proved that the percentage of mammary tumours in the castrates of this strain is higher than in normal males. The results in the hybrids indicate that the incidence is higher in the castrated animals. In the dB-group the difference is significant, but in the dO-group the significance is doubtful.

Whole mounts of the d-strain were examined to determine whether the mammary gland had developed in œstrone-treated castrated males. Seven of the 11 glands examined had developed well. Despite a better development of the mammary gland, the frequency of tumours was not higher.

The next experiments were designed to find a treatment which gives a higher incidence of mammary tumours in

castrated d-males. Firstly we tried progesterone. I think it is agreed at present that progesterone alone may also stimulate the development of mouse mammary gland. I believe that Gardner was the first to prove this some years ago. It is also agreed that progesterone requires much higher dosage than does œstrone. Using the method already mentioned, the threshold value for the first signs of mammary growth in castrated males of the d-strain was determined. Progesterone provokes the same first signs of growth as œstrone does. *The threshold value for progesterone was 500 μg.*, which is 2,500 times that of œstrone (0·2 μg.).

A combination of œstrone and progesterone was tried in castrated Dilute-brown males so as to determine the possibility of producing better mammary development, i.e., a higher tumour frequency (Table IV).

Table IV

MAMMARY-TUMOUR-INCIDENCE IN D MALES CASTRATED AND IMPLANTED WITH ŒSTRONE+PROGESTERONE PELLETS

	No	*No. living at tumour age*	*Percentage mammary tumour incidence*	*Average tumour age Months*	*Non-tumour age Months*
♂d	32	24	4	14	18
Mammary development in 10 whole mounts					
No growth		*Little growth*		*Developed*	
0		2		8	
With Progesterone only					
	No	*No living at tumour age*	*Percentage mammary tumour incidence*	*Average tumour age Months*	*Non-tumour age Months*
♂d	71	58	0	—	16
Mammary development in 19 whole mounts					
No growth		*Little growth*		*Developed*	
1		18		0	

Table IV shows that the expected rise was not produced. It is doubtful if the mammary gland developed better than after œstrone alone. It is therefore not surprising that implantation of pure progesterone-pellets alone remained without effect. Mammary development was only slight.

In an experiment carried out by Korteweg many years ago, in which ovaries were grafted into castrated males of the d-strain, the cancer percentage was higher than after hormone treatment. This experiment was repeated (Table V).

Table V

	No	*No living at tumour age*	*Percentage mammary tumour incidence*	*Tumour age Months*	*Non-tumour age Months*	*Percentage still living*
♂d	37	36	64	13	13	22

Dilute-brown males were castrated and grafted with ovaries of their sisters at the age of one month. The cancer percentage was shown to correspond with that of untreated females.

Thus, hormone treatment under the conditions of our experiments cannot equal the effect of grafted ovaries in males of the d-strain.

Hormone treatment in the experiments mentioned so far was started at the age of one month, as it was assumed that the earlier hormone treatment is started, the higher is the frequency of the mammary tumour. The next experiments were done to test the influence of the age at which the hormone treatment was started.

Table VI shows the results in normal males treated at the age of 6 and 12 months. In the d-strain, of course, no results can be expected. Comparison of tumour frequency in hybrids treated at one and six months shows the percentage to be little lower when treatment is started later. The average tumour age is later, as was clearly demonstrated in hybrids dO.

Table VI

NORMAL MALES TREATED WITH ŒSTRONE

	Beginning treatment at Months	*No*	*No living at tumour age*	*Percentage mammary tumour incidence*	*Tumour age Months*	*Non-tumour age Months*
♂d	6	25	10	10	16	21
♂d	12	63		0		19
♂d	1	78	44	4	14	17
♂dB	6	41	38	16	17	20
♂dB	1	20	19	21	15	21
♂dO	6	25	18	33	17	22
♂dO	1	27	20	40	10	16

Table VII gives the results of treatment of castrated males at a later age. Of course nothing can be expected from the treatment of d-males. But neither does ovarian grafting at the age of 12 months give rise to tumours. In this experiment the d-males were castrated at the age of one month and at the age of 12 months ovaries were grafted from one month old d-females. These animals showed good mammary

Table VII

CASTRATED MALES TREATED WITH ŒSTRONE

	Beginning treatment at Months	*No*	*No living at tumour age*	*Percentage mammary tumour incidence*	*Tumour age Months*	*Non-tumour age Months*
♂d	6	82		7	18	19
♂d	12	21	—	0		18
♂d grafted with ovaries	12	20	—	0		18
♂dO	6	28	24	29	18	20
♂dO	1	21	21	71	12	24
♂dB	12	38		0		23
♂dB	1	8	8	75	15	20

development, often with hyperplastic nodules, but no mammary cancer was observed. In the group hybrids dO, castrated and implanted with hormone pellets at the age of six months, the tumour percentage found was 29 per cent, as against 71 per cent found when treatment was begun at one month. The average tumour age was six months later. Treatment begun at 12 months gives no cancer in dB-hybrids. Thus treatment begun at a later age than one month results in a decrease in the percentage of tumour incidence.

In the next experiment treatment was started as early as possible, i.e., on the first day of life.

There was no difference in the cancer incidence with treatment begun on the first day of life and treatment begun at one month in female d (see Table II). Table VIII gives the

Table VIII

FEMALE AND MALE Od FOSTERED BY FEMALE d FROM THE EIGHTH DAY OF LIFE

		No	*No living at tumour age*	*Percentage mammary tumour incidence*	*Tumour age Months*	*Non-tumour age Months*
♀litter mates	untreated virgins	32	32	100	13	
	œstrone from the first day	26	11	91	11	9
	œstrone at the age of one month	27	26	89	11	20
♂litter mates	♂ impl œstrone first day of life	24	13	62	8	18
	♂ œstrone one month	17	17	71	11	23

results in male and female hybrids Od. These hybrids are free from the mammary-tumour-agent, therefore they were fostered by a d-female from the eighth day of life. One of three female littermates remained untreated; the second was

treated with œstrone pellets on the first day, and the third with œstrone at weaning age. The same treatment was carried out with male littermates; the first group was treated with œstrone from the first day; the second group received œstrone at the age of one month. Both groups of males were castrated at the age of one month. There was no difference in tumour incidence between the various groups. Comparison of the tumour-age in the females shows no difference whether treatment is begun at the first day or at the first month. Whole-mount preparations of the mammary gland in the females treated on the first day with œstrone showed no development after three weeks; in fact, there was little if any difference with the mammary glands of untreated females of the same age. Perhaps the administration of œstrone to these very young animals gives no mammary development because the hypophysis is not yet functioning.

In the males there is a difference in the average tumour-age between the two groups. Whole-mounts show that there is in the treated group some slight mammary development, which of course is lacking in the untreated males at one month of age.

It remains unexplained why there is a difference in the average tumour age between females and males treated on the first day of life. This matter is still under investigation.

This is a short review of the results of some of our experiments. These results confirm that steroids have an effect on the development of mammary-tumours in mice, but they clearly show that a variety of factors have to be taken into account in the evaluation of these effects.

DISCUSSION

GARDNER: Did you implant just one pellet of œstrone and cholesterol and one pellet of progesterone? How long did these pellets last in the animal?

MÜHLBOCK. Œstrone and cholesterol lasts 1½–2 years, but progesterone varies. After some months you cannot find the progesterone pellets.

BEGG: Have you any evidence that there was still œstrone in the pellets, not just cholesterol?

MUHLBOCK· Yes; after one and a half years I have extirpated the pellets and put them into castrated female mice, and there was mammary growth.

FOLLEY: You used pure progesterone?
MUHLBOCK: Yes.

from the pellets.

MUHLBOCK: But they had been getting some progesterone, because we found development of the mammary gland in practically all of the animals.

FOLLEY: You might get the beginning of development, but you wouldn't get full mammary development unless you had a pretty large dose of progesterone.

of the goat with pellets to get enough progesterone uptake to have any effect on the udder. That's why we have to use injections to administer amounts of about 40 mg. a day.

BEGG How does a tiny pellet last so long?

HERTZ The cholesterol holds it up. What is the concentration of the œstrogen?

MUHLBOCK 25 per cent. I tried some other concentrations, but if

to six weeks.

FOLLEY We have found that 50 mg. pellets of œstradiol in humans will last for over a year.

working as well.

DMOCHOWSKI: I was interested in the very low incidence of mammary cancer in males of strain d In connection with what Dr. Pullinger just mentioned, as we now know that males of *high-cancer strains* contain the milk factor in their tissues, and that they are susceptible,

then it is the third factor, the hormonal, which does not seem to be effective. Have you any suggestions why the incidence was so low? As far as I am aware, no experiments have been carried out comparing the amount of milk factor in males of various high-cancer strains. [illegible] Perhaps the rate of elimina- [illegible]

experiments carried out in the United States and in this country, the incidence of mammary cancer following œstrone treatment is always very high in males of the high cancer strain and comparable to the incidence in females of the same strain.

MÜHLBOCK: I will deal with your second point first. In the hybrids I found practically the same incidence in females and treated males. However, Dr. Bonser, who has treated A Strong male mice, has found only one or two mammary cancers after treatment. I think that probably in this d-strain the margin between the effective dose and the toxic dose is too narrow. Maybe I would get a higher incidence if I gave more œstrone, but the mortality would be still higher than it is with 25 per cent.

HUGGINS. To what do you attribute the increased efficiency of the [illegible] in producing cancer in the d males? [illegible]

DMOCHOWSKI I am not aware that this has been done, but [illegible] see any reason why they should be inactivated.

HADDOW: *You have assumed, Dr. Muhlbock, that the influence is not direct?*

MÜHBOCK: Yes. Mammary-tumour-agent is present in castrated and in pregnant female mice, although there is much more hormone in the latter.

ASTBURY: But no one has simply tested an extract of these virus particles to see if they're directly influenced chemically by these hormones?

[illegible] œstrone is [illegible] ld [illegible] ay [illegible]

GARDNER: Yes, that is the hypothesis.

[illegible]

uniquely different from the other mice that were mentioned in that they do show adrenal tumours after the gonads have been removed for a long time, whereas I know the C_{17} does not. In the males of the ce strain, they have occasionally found a mammary tumour as well as the adrenal tumour.

MÜHLBOCK: Our subline of the DBA strain does not show any adrenal tumours.

HORMONAL RESPONSES OF MAMMARY TUMOURS IN MICE

L. F. FOULDS

I BECAME involved in the study of mammary tumours by following a procedure designed specifically to avoid them. I bred hybrid mice, using C_{57} black females and R III males, and found an appreciable number of tumours in the F_1 hybrids although they ought not to have had any milk factor from their C_{57} black mothers. I thought it might be interesting to see if there was anything peculiar about the tumours as shown by transplantation, and I made a few experiments to see if milk factor was present.

I will pass over these second experiments very quickly. They show that milk factor is present. Where it comes from is another matter, which I don't propose to go into. I should like to emphasize now that all the mice to which I shall have occasion to refer this morning, so far as all the evidence goes, do possess the Bittner milk-factor.

One of the experiments consisted in breeding from those F_1 mice which developed mammary tumours. I have inbred from some of those mice now for 12 or 13 generations, and the offspring provide many of the tumours which I shall describe. The remaining mice are the reciprocal hybrids of the F_1 generation, that is, they have R III mothers and C_{57} black fathers. Most of the observations have been made on one or other of these hybrids.

The tumours, when transplanted, grew very well in normal female mice. They did not grow at all, or they grew only after a long delay, in intact male mice and they did not grow in castrated male mice. In males which received pellets of stilbœstrol in cholesterol, the tumours grew as well as in normal females. In one or two experiments the pellets were

removed from males after the transplanted tumours got well going. Sometimes there was a slight arrest of growth, but it was temporary, and eventually the tumours went on growing. So the action of œstrogen seemed to be mainly on the initial take of the tumour rather than on its continued growth.

There were two other bits of evidence for hormonal action on these tumours. Some of them were very conspicuously milky. When they were cut they oozed a milky or even creamy fluid from their surface and they often had milky cysts. This was found only in pregnant females or in œstrogenized males; in males from which the œstrogen pellets had been removed the tumours were no longer milky. It occurred in a minority of the tumours which showed the difference in transplantability in males and females. The second point is that tumours of two strains grew during pregnancy and tended to regress, though not completely, after parturition, which is the reverse of the usual experience of the effect of pregnancy on transplanted tumours. One other thing I should say about these phenomena is that they were temporary; when one went on transplanting, then sooner or later the tumours grew equally in males and females. Sometimes this occurred after the first or second passages, sometimes after half a dozen or more.

When the experiments on transplantable tumours had got to this stage, spontaneous tumours began coming up in the breeding experiments, and I did not follow up the transplantation experiments but concentrated on spontaneous tumours. So the tumours I am going to describe now are spontaneous tumours growing in their original hosts.

When tumours began appearing in the breeding experiment, it was noticed that a tumour found one week couldn't be found the next week. When that had happened a few times I looked into the matter and found that about two-thirds of the tumours grew during pregnancy and regressed very rapidly after parturition. The tumours were measured regularly, and their size was plotted against time. The course of the familiar type of spontaneous mammary tumour, which

I call "unresponsive," as it grows without any regard to pregnancy or parturition, was represented by an approximately straight line. The other tumours, which were in a majority and which I call "responsive" tumours, were represented by waves whose peaks corresponded approximately with the times of parturition in successive pregnancies. There were two main types of "responsive" tumour. In one type, the tumours reached about the same peak size in successive pregnancies and regressed, often completely, between pregnancies. In the other type, the peak size increased steadily in successive pregnancies and regression between pregnancies was sometimes complete, but often only partial. Regression after parturition was often remarkably abrupt. Tumours of substantial size (e g., $1 \cdot 5 \times 1 \cdot 0$ cm.) often disappeared within 24 hours. The responsiveness sometimes persisted through many pregnancies over a long time. Thus, in a mouse under observation for 21 weeks, one tumour reached the same size in six successive pregnancies and regressed after parturition, whilst another grew gradually, showing a little fluctuation about the time of each parturition. Sometimes there was a much bigger swing and tumours reached a substantial size, the maximum being about 2·5 cm. in diameter, whilst still responsive to pregnancy. If breeding were stopped by removing the male, the tumours disappeared and, as a rule, they did not come back again until the male was brought back and breeding resumed. Then they promptly reappeared. *Some tumours maintained the same type of behaviour* throughout their clinical course, but others changed their course often abruptly; in particular, tumours which previously had grown only during pregnancy lost their dependence on the stimulus of pregnancy and then grew steadily and progressively without regard to reproductive activity. When, as was common, multiple responsive tumours were present, the change to unresponsive behaviour occurred in only one of them. For example, two tumours appeared simultaneously in one mouse and a third developed later. The three tumours followed parallel courses, growing during pregnancy and

regressing thereafter until the ultimate pregnancy; then the three tumours grew parallel up to the time of parturition, whereupon two tumours promptly regressed, whereas the other continued to grow steadily. The tumour that continued to grow was one of the pair that had appeared first. I think that this and other similar observations can only mean that a change occurred in one of the tumours; had the whole animal changed, all the tumours would have been affected equally. I have described the change as "progression" by which I understand an irreversible qualitative change in the tumour itself, whereby the tumour, previously responsive to pregnancy, becomes unresponsive and grows progressively until it kills the animal, whether the animal breeds or not. Sometimes a tumour does recur in the absence of pregnancy. For example, the male was removed from a mouse with three tumours and no more pregnancies occurred during the period of observation. The three tumours disappeared; one of them recurred after three weeks and grew steadily and progressively without any stimulation. That tumour had changed from the responsive to the unresponsive type, irrespective of pregnancy. My suspicion is that, in general, pregnancy is not essential for the occurrence of progression although it often brings it to light.

I can't go into details very closely, but the peak of growth was reached, as a rule, during the last two or three days of pregnancy. Regression began, I suspect, a little before the actual time of parturition—it is difficult to be certain—but it was pronounced within the first 24 hours. Recurrence was usually obvious during the second half of the next pregnancy, but I think it probably started considerably before then, and there were some indications that the recurrence possibly started during the first week of pregnancy.

One of the main problems to be followed up is: what is the hormonal mechanism involved? My colleague, Mr.

I call "unresponsive," as it grows without any regard to pregnancy or parturition, was represented by an approximately straight line. The other tumours, which were in a majority and which I call "responsive" tumours, were represented by waves whose peaks corresponded approximately with the times of parturition in successive pregnancies. There were two main types of "responsive" tumour. In one type, the tumours reached about the same peak size in successive pregnancies and regressed, often completely, between pregnancies. In the other type, the peak size increased steadily in successive pregnancies and regression between pregnancies was sometimes complete, but often only partial. Regression after parturition was often remarkably abrupt. Tumours of substantial size (e.g., $1 \cdot 5 \times 1 \cdot 0$ cm.) often disappeared within 24 hours. The responsiveness sometimes persisted through many pregnancies over a long time. Thus, in a mouse under observation for 21 weeks, one tumour reached the same size in six successive pregnancies and regressed after parturition, whilst another grew gradually, showing a little fluctuation about the time of each parturition. Sometimes there was a much bigger swing and tumours reached a substantial size, the maximum being about $2 \cdot 5$ cm. in diameter, whilst still responsive to pregnancy. If breeding were stopped by removing the male, the tumours disappeared and, as a rule, they did not come back again until the male was brought back and breeding resumed. Then they promptly reappeared. Some tumours maintained the same type of behaviour throughout their clinical course, but others changed their course often abruptly; in particular, tumours which previously had grown only during pregnancy lost their dependence on the stimulus of pregnancy and then grew steadily and progressively without regard to reproductive activity. When, as was common, multiple responsive tumours were present, the change to unresponsive behaviour occurred in only one

bulbous extremities. There is usually a lumen in the columns, but, as a result of the very great epithelial proliferation, the lumen sometimes becomes quite inconspicuous. At the height of their growth these epithelial masses contain many mitotic figures, and they are separated from each other by a very cellular, very loose and vascular connective tissue with no collagen. Soon after parturition a breaking down of these epithelial masses is seen. The tubes are now lined only by a single layer of epithelium. The bulk of the epithelium is desquamated into the lumen. As I can't find much sign of phagocytosis or cellular reaction either in or around these tubules, I presume that the desquamated masses are extruded by way of the mammary ducts. When the epithelial degeneration begins, the connective tissue also changes and collagen forms between the epithelial columns. Two or three days after parturition, we find empty tubules with a single-layered epithelial lining, and a certain amount of shrinkage, compression, and collapse of the tubules and fibrous collagenous connective tissue between them. This process goes on to produce a nodule with a peripheral part of quite dense fibrous tissue with collapsed, compressed, epithelial tubules and often a central area of loose fatty tissue containing some ducts.

Many of the tumours which regressed after parturition disappeared completely; one couldn't find them either by the naked eye or with a dissecting microscope. What happens to those I cannot say, but possible transitions were found. For example, at the site of a tumour which had regressed in a mouse which had not been pregnant for about three months previously, there was a fibrotic plaque which was obviously incomplete; in the animal it looked a distinct well-outlined plaque; histologically, patchiness of the fibrous area was conspicuous. In another mouse whose tumour had regressed about seven months previously, one could see by the naked eye a fairly distinctly outlined plaque, but histologically there was only a thin rim of fibrous tissue around the edge, enclosing tissue resembling normal breast. It is possible to imagine this process going on a little further so that one would

against in dosage, and the balance in dosage of different hormones. So far, we have no clear indication that it is either a purely œstrogenic effect or a progesterone effect, or even a combination, although I must admit we haven't enough information with balanced doses to draw firm conclusions.

The unusual behaviour on transplantation and the growth in relation to pregnancy were quite unexpected and raised the question whether the tumours differ in some way from those with which we are familiar in most mice. The final big tumour which kills the mouse, so far as I can see, is no different from the ordinary mammary tumours of inbred or cross-bred mice which have been described in the last 40 or 50 years. The early stages do seem to be different. Unfortunately, it was not possible to keep the histological work in line with the experimental observations or to do whole-mounts on the breasts, and, to tell the truth, I did not foresee at the time how useful they might have been. All that I can say now is that the hyperplastic nodules which have been described by Dr. Gardner and others, and which are common in most high tumour strains, are, at least, not conspicuous in these mice, and I don't think the tumours develop from them. The first sign I see is a flat disk or a plaque in the subcutaneous tissues. A characteristic feature is that there are usually one or more fairly normal-looking ducts in the centre, sometimes even a fairly distinct division between a "medulla" and a "cortex," with tubules often radiating from the centre towards the periphery. Some bigger tumours are made up of several lobules of the kind which make up the small one. The histological appearances are extremely varied and complicated and I do not pretend to have analysed any but the simpler ones.

There is one kind of tumour, however, in which the sequence of changes is, I think, fairly convincing. The small tumours near the end of pregnancy and at about the peak of growth, have a centre occupied by small ducts and columns of epithelial tubules radiating outwards. There is a certain amount of branching of these radiating columns, which often have

It seems to me that the essential histological feature of these tumours is that the type of growth is essentially organoid; it is not a mere cell proliferation. The growths imitate, to a greater or lesser extent, the development of the normal breast and, to some extent, though not exactly, they react to similar hormonal conditions. I would point out one difference, that immediately after parturition the responsive tumour is quite out of step with the surrounding normal breast: the normal breast is full of secretion and active whereas the tumour is already, within a few hours of parturition, well on the way to disappearance.

I rather suspect that the phenomena I have described may correspond to something which occurs only occasionally in other strains of mice; what is rare in most strains is the usual and commonplace feature in these particular hybrid strains. I am less interested in their application to the particular problems of mammary tumours in mice than to the more general problems of responsiveness to hormones and progression from a responsive to an unresponsive state, both of which I think are highly relevant to our discussion to-day.

DISCUSSION

HADDOW: There is no doubt this is a very important observation. [illegible]

pregnancy, whereas in any case it would have appeared about that time.

FOULDS. The time of recurrence in our mice was quite erratic. It varied by some months.

HADDOW: Willis, I think, described a case of the development of a mammary cancer in a young woman during pregnancy. For other reasons, the pregnancy was terminated and the mammary carcinoma completely regressed and remained absent until a subsequent pregnancy in which it reappeared.

not be able to see anything at all by the naked eye. In early pregnancy, the sequence of changes which I described for regression goes in the reverse direction: the tubules begin to swell up, the fibrous tissue loosens, the collagen disappears and the tumour reverts to the active plaque which I described before.

Most of these tumours regress whether or not the mouse nurses its young. A few, however, do not regress while the animal is nursing, though they may regress later, and some at least of these during the nursing period have the structure of secreting adenomas. There is also a trace of the architecture which I described in the other tumours, with branching tubular duct-like formations.

There is a little evidence about the mechanism and the histological basis of what I call "progression," that is the change of properties of the tumour during observation. In some regressed tumours the regression is patchy, with survival of some small patches of epithelial tubules which have not regressed in step with the bulk of the nodule; or whilst the bulk of a tumour is degenerate and becoming fibrous, one lobule is obviously different and retains the structure which we normally find during pregnancy. This I interpret as altered tissue which probably goes on to form a progressively growing, unresponsive tumour. In some regressed plaques there is sometimes a central, sometimes a peripheral nodule of what is recognizable as fairly typical mammary tumour. These nodules again, I think would have given rise to a progressively growing, unresponsive tumour. The fact that these unresponsive tumours become apparent very often at the end of a pregnancy may, I think, be due to a vascular effect. The nodules from which they start in non-pregnant mice are enclosed sometimes in a quite dense fibrous tissue and may have a very poor blood supply. When pregnancy occurs and the whole surrounding tumour becomes better vascularized, then the nodules get going, and once they have acquired a good blood supply they continue to grow indefinitely.

closely related to Compounds E and F. On the other hand, there appear in the urine some new steroids whose nature is not known.

What experience has been accumulated on œstrogen treatment of human males? Somebody mentioned that there are more than half a dozen males with prostate cancer who have developed breast tumours under œstrogen treatment.

HADDOW: I think there are four papers describing development of breast cancer in the male after œstrogen treatment.

HUGGINS: It is not certain whether it's breast cancer or prostatic cancer which has metastasized to the breast.

and cervical cancer. Perhaps a number of these people who have been treated with œstrogens for prostatic cancer clinically presented their prostatic cancer before they presented their second primary breast cancer. I don't think you can jump to the conclusion that there is a cause-effect relationship.

The bone metastases did not have a high acid phosphatase content.

as to the recurrence of these tumours. For the last two years we have had an experiment set up on similar lines to those of Dr. Foulds.

FOULDS: One transplanted tumour

Its presen

offspring

incidence

GARDNER: I have been extremely interested in this work which Dr Foulds has described. On one occasion I saw, I think, three tumour ... after parturition And ...

tissue. How does the metabolism differ from that in the mammary glands that do not disappear? The histological appearance seems to be the same as in human tumours that disappear under œstrogen treatment.

HERTZ: It is paradoxical that the histology of tumours disappearing under intensive œstrogen administration should be compared to *these*, which are regressing in the period of withdrawal of the high hormonal level of pregnancy.

HADDOW: We had a few very striking cases at the Cancer Hospital of very active clinical regression on withdrawal of œstrogen.

HERTZ: We've seen that in cases that I'll talk about tomorrow, in which we continued to get very marked degrees of regression after stopping œstrogen in older patients with breast cancer who were no longer responding to therapy.

BEGG: Clinically, the œstrogen regression would depend on whether they are pre- or post-menopausal, wouldn't it?

HERTZ: That is a generalization that has been made, but I don't know if it's too well established What do you think, Dr. Haddow?

HADDOW: I think it's part of the truth, but not all. In the early days we had a joint clinical trial by about 12–14 people, all of whom worked independently for about a year and then came together. About half of these investigators had had this very distinct impression, and when they all came together it seemed to be much more than that, although not the whole truth.

BEGG: Won't a woman with breast cancer have a flare-up during pregnancy?

HADDOW: Apart from any specific effect, the question of blood supply is important.

DOBRINER: Does testosterone have any effect on the tumours?

FOULDS: We haven't tried testosterone. The sort of experiment we've been doing is to remove the males, let the tumour regress, and then try to bring it back artificially.

DOBRINER: It is very interesting that you have a host-tumour ... is dependent on the hormonal environ- ... nal changes in mice ... there are two major

PART III

STEROIDS IN CANCER THERAPY

ANTI-TUMOUR ACTIVITIES OF STEROIDS IN ANIMALS

C. CHESTER STOCK

THIS report includes the studies of several of my associates in the Experimental Chemotherapy Division of the Sloan-Kettering Institute and represents only one of a number of investigations on steroids being conducted at Sloan-Kettering. The Chemotherapy Division programme* includes tests of a number of steroids against the chick embryo by Dr. Karnofsky (Karnofsky, Stock and Rhoads, 1950), against a variety of transplantable tumours in mice and rats by Dr. Sugiura (Sugiura, Stock, Dobriner and Rhoads, 1950), and against mouse leukæmia by Dr. Burchenal (Burchenal, Stock and Rhoads). The mouse leukæmia studies will be presented by Dr. Burchenal at this conference. Clinical and metabolic studies will be reported by Dr. Dobriner. Other aspects of the Institute's biological studies on steroids include Dr. Woolley's investigations on the influence of steroids on organs and tumours in mice, and Dr. Money's studies on steroid-induced changes in the rat and the influence of steroids upon the uptake of radioactive iodine by the thyroid.

The classical experimental and clinical studies of the influence of steroids on tumours by Gardner (1947), Huggins (1942-1946), Nathanson (1947), Haddow (1944), and Adair

*We wish to acknowledge the valuable assistance of Dr. Konrad Dobriner in this programme.

incidence is 70 per cent [illegible]

carry milk factor obtained somehow or other, and the tumour-free F_1 animals, which are in the majority, do not.

DMOCHOWSKI: I think that the picture is even more complicated. I believe that our observations agree with those of Dr. Andervont from Washington, that not all of these tumours, as far as biological tests are concerned, contain the milk factor. We have 20 at the moment that are being tested biologically, and I can say for certain that some of [illegible]

[illegible] test mice. I believe that in this type of breast tumour [illegible]

breeding. It seems to us that forced breeding may produce two types of tumour, one which contains the milk factor, and the other which does not seem to possess it. Experiments are being carried out at the moment to elucidate this point.

(1946, 1949) are well known. These and other published reports have been excellently supplemented by the studies presented at this conference.

Our results with cortisone were foreshadowed by those of Heilman and Kendall (1944) on the inhibition and regression of a mouse lymphosarcoma, and by Murphy and Sturm (1944) on the inhibition of the development of transmitted leukæmia in rats by adrenal cortical extracts. Clinical studies with cortisone and ACTH were under way in patients (Pearson, Eliel, Rawson, Dobriner and Rhoads, 1949) at the time our experiments with steroids on transplanted tumours in mice and rats were initiated. We were interested in determining:—

(1) Whether the lymphosarcomas in our chemotherapy programme, under the test conditions employed, would respond to cortisone.
(2) *Whether types of tumours other than those of lymphoid origin would be affected.*
(3) *Whether there are differences in patterns of activity of different steroids on different tumours.*

It was soon apparent that some steroids would be available in amounts too limited even for preliminary trials in the animal. As a result, studies were initiated in the chick embryo and, since cortisone showed a marked activity against *the embryo, all available steroids have been screened in the* chick embryo (Karnofsky *et al.*, 1950). The experiments with cortisone revealed such striking effects that a discussion of them and the results with other steroids seems warranted prior to a discussion of the activities of steroids against abnormal growth.

Landauer (1947) first reported that an extract of adrenal cortex would inhibit the growth of the chick embryo. *In Karnofsky's experiments cortisone acetate has exerted a marked inhibitory effect on the growth of the chick embryo. Initially, aqueous suspensions of 2–4 mg. of steroid were* injected into the yolk sac of the four-day-old embryo. The

embryos surviving to 18 days showed moderate to severe stunting with generalized baldness. The effect was explored in more detail and a range of growth inhibition was found. The range of effects has been divided into four categories as illustrated in Fig. 1. The embryos dying before the 18th day are small, pale and œdematous, have unusually large eyes in relation to body size, and are often eviscerated. Those surviving to 18 days present a characteristic syndrome. The embryo is small; the yolk sac and chorioallantoic membrane have not formed completely, and there is consequently free yolk and albumin present which is not enveloped by membranes. The embryo adopts a characteristic, curved position and the amnion is drawn tightly around it. The embryo is pale and the feathers are practically absent; evisceration is common. In Karnofsky's experience other types of chemical agents have not produced a similar stunting. At lower doses are seen intermediate changes, consisting of diminished feather formation with baldness, clubbed feathers, and varying degrees of stunting in body growth. Embryos showing the intermediate effects may survive until 21 days but they do not hatch.

The effect of cortisone on the embryo was quantitated at four days and at eight days for yolk sac injections and at eight days for the chorioallantoic route of administration. In the latter the maximum effect was obtained with 2 mg. of cortisone acetate and larger amounts were required by the other route (Table I). The relationship of time of dosage to the effect was studied and the data in Table II indicate that inhibition has been obtained by both routes of administration until the 12th day. The physiological significance of this observation may be clarified by histological studies still in progress.

The assay of steroids for cortisone-like activity has been developed as follows:—

The steroid solution or suspension is placed on the chorioallantoic membrane of the eight-day-old developing chick embryo. Ten days later the embryo is sacrificed and graded

To face page 137]

CONTROLS

STAGE I

STAGE II

STAGE III

STAGE IV

SACRIFICED, 18 DAYS INCUBATION

WGT. 16–21 G

SLIGHT FEATHER CHANGES

SLIGHT STUNTING

USUAL WGT 13–16 G

MODERATE BALDNESS CHIEFLY ON HEAD

CLUBBED FEATHERS

MODERATE STUNTING

USUAL WGT 10-14 G

SEVERE GENERALIZED BALDNESS

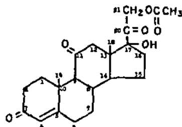
SEVERE STUNTING

USUAL WGT 8-11 G

"CORTISONE SYNDROME"

FIG. 1 Range of effects produced by cortisone in the chick embryo

for effect. With this technique a number of steroids have been tested with the results presented in Figs. 3, 4, 5 and 6. Fig. 3 shows the approximate minimal amounts of compounds E, F, A and corticosterone needed to achieve definite inhibition of the embryo. The greater effectiveness of Compound F is striking. In Fig. 4 the activities of Compound S and DCA



11-DEHYDRO-17-HYDROXYCORTICOSTERONE ACETATE

FIG. 2. Formula for cortisone showing numbering system for reference on steroids listed in the tables.

H₂C-OH C=O OH

11-DEHYDRO-17α-HYDROXY CORTICOSTERONE
COMPOUND E

ACETATE + 0.5 mg.
FREE

H₂C-OH C=O OH HO

17α HYDROXY CORTICOSTERONE
COMPOUND F

+ 0.02 mg.

H₂-C-OH C=O

11-DEHYDRO CORTICOSTERONE
(KENDALL'S COMPOUND A)

ACETATE + 0.2 mg
FREE

H₂-C-OH C=O HO

CORTICOSTERONE

+ 0.2 mg

FIG. 3. Formulæ of compounds with approximate minimal amounts required to inhibit the development of the chick embryo.

Table I

AVERAGE RESULTS OF EFFECTS ON CHICK EMBRYO FROM VARIOUS AMOUNTS OF CORTISONE ADMINISTERED BY SEVERAL ROUTES
ACTIVITY OF CORTISONE ACETATE IN THE CHICK EMBRYO

Dose mg/egg	4-day yolk sac		8 day yolk sac		8 day chorioallantois	
	Embryo effect (1–4, average)	Weight, g (average)	Embryo effect (1–4, average)	Weight, g (average)	Embryo effect (1–4, average)	Weight, g (average)
	(Sacrificed at 17–18 days)					
0·2					0(3)	16·9
0·5	+0 8(5)*	14 5	0(4)	17·4	+2·7(7)	10·9
1 0	+1 3(21)	13 0	+1 0(4)	13 8	+2·5(2)	10·5
2 0	+2 4(19)	10 8	2 8(11)	8 8	+4·0(8)	5·2
4 0	+4·0(3)	10 1	4·0(3)	4·4		

*Number of embryos.

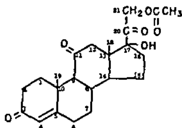
The effects are graded from 1 to 4 as presented in Fig 1.

Table II

ACTIVITY OF 2MG/EGG OF CORTISONE ACETATE INJECTED INTO THE YOLK SAC AND ON CHORIOALLANTOIC MEMBRANE (0–15 DAYS OF AGE)

Time of injection Days	Route			
	Yolk sac		Chorioallantois	
	Embryo effect (1–4, average)	Weight, g. (average)	Embryo effect (1–4, average)	Weight, g (average)
	Sacrificed 17–18 days			
0	+2·1(6)†	10·0	—	—
2	+2 1(6)	11·3	—	—
4	+1 8(7)	10·7	—	—
6	+2 1(5)	10·0	—	—
8	+2·8(8)	8·4	+4·0(6)	5 2
10	+2 2(9)	10·0	+3 8(4)	7·4
12	+3 1(14)	7 1	+2 6(9)	7·8
15	0(9)	17·5*	0(9)	15 8*
Controls	(16 to 21 g.)			

*Sacrificed, 19 days
†Number of embryos
The effects are graded from 1 to 4 as presented in Fig 1



11-DEHYDRO-17-HYDROXYCORTICOSTERONE ACETATE

FIG. 2. Formula for cortisone showing numbering system for reference on steroids listed in the tables

for effect. With this technique a number of steroids have been tested with the results presented in *Figs.* 3, 4, 5 and 6. Fig. 3 shows the approximate minimal amounts of compounds E, F, A and corticosterone needed to achieve definite inhibition of the embryo. The greater effectiveness of Compound F is striking. In Fig. 4 the activities of Compound S and DCA

11-DEHYDRO-17α-HYDROXY CORTICOSTERONE
COMPOUND E
ACETATE + 0 5mg.
FREE

17α-HYDROXY CORTICOSTERONE
COMPOUND F
+ 0 02mg

11-DEHYDRO CORTICOSTERONE
(KENDALL'S COMPOUND A)
ACETATE + 0 2 mg
FREE

CORTICOSTERONE
+ 0 2mg

FIG. 3. Formulæ of compounds with approximate minimal amounts required to inhibit the development of the chick embryo.

are compared. It is of interest that DCA has a definite activity although it lacks an 11-oxygen function. The markedly decreased activity of Compound S is worthy of note. With progesterone (Fig. 5) there appears to be little difference due to the presence of the 17-OH group, nor has the activity been lost with the loss of the 21-OH group. In

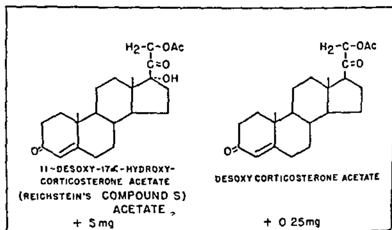


FIG. 4.

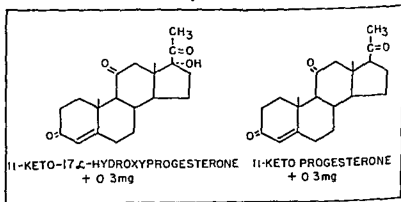


FIG. 5

Fig. 6 it appears that several compounds of the progesterone and Δ^5-pregnenolone series have an activity quantitatively similar to that of Compound S. Over twenty other steroids have been tested at 5 or 10 mg. levels without showing any

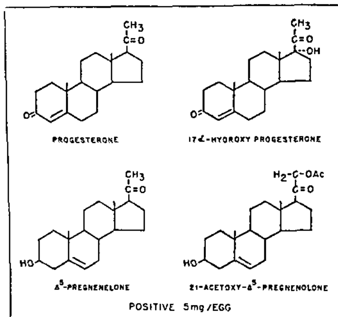


FIG. 6.

FIGS. 4, 5, and 6. Formulas of compounds with approximate minimal amounts required to inhibit the development of the chick embryo.

inhibition of the embryos. In Table III these are listed by name and grouped according to certain common aspects of structure.

In addition, a group of C_{19} steroids were without inhibitory effects. Included were testosterone, adrenosterone, Δ^4-androstenediene-3,17, 17-ethinyl testosterone, $\Delta^{4,6}$-androstadiene-3,17 and 2-acetoxy testosterone acetate.

Table III

STEROIDS INACTIVE AS TESTED AGAINST THE CHICK EMBRYO

Compound	Max. dose level tested, mg /egg
Compounds with an 11 oxygen function and a saturated A and B ring	
11-keto-pregnanolone acetate	— 5 0
3, 11, 20-triketo-4, 21-diacetoxy,17-OH pregnane	— 5·0
Dihydro-cortisone acetate	—10·0
Compounds without an 11 oxygen function and a saturated A and B ring	
Allo-pregnanolone	—10 0
Allo-pregnanediol, 3β, 17α-one-20-acetate 3 (Comp L)	— 5·0
Pregnane-3α, 12α, 21-triol-2p-one, 21 acetate	— 5·0
A compound without an 11 oxygen function and an unsaturation at C_1	
Δ^{1}-17 hydroxyprogesterone	— 5·0
Compounds without an 11 oxygen function and no carbonyl group at C_{20}	
[illegible]	— 8 0
[illegible]	—10·0
[illegible]	— 7·5
[illegible]	— 5 0
[illegible]	— 5·0
[illegible]	— 5·0
[illegible]	— 5·0
A compound without an 11 oxygen function and unsaturated at C_{16}	
$\Delta^{5,16}$-pregnadiene-3β-ol-20-one acetate	— 5 0

To sum up, certain steroids have been found to inhibit chick embryo development at the following levels:—

·02 mg.	Compound F
0·2–0·5 mg. . . .	Corticosterone
	Kendall's Compound A
	Cortisone
	11-ketoprogesterone
	11-keto-17-hydroxy-progesterone
	DCA

5 mg. . . . . . Reichstein's Compound S
progesterone
pregnenolone

The effects observed differ from those caused by X-rays and those caused by the antagonists of folic acid and other vitamins; however, the exact nature of the activity is yet to be determined. It is believed that this will offer a useful tool for detecting small amounts of certain biologically active steroids, though the steroids active against the chick embryo may not duplicate those showing any other type of biological activity. It will be seen that the number of steroids active against certain mouse tumours is more restricted than the number active against the chick embryo.

The studies of the action of steroids against the chick embryo are being extended to include newly hatched chicks (2–3 days). So far, there have been observed inhibitions in weight gain, measured at 10 days, a pronounced effect by compound F, slight effects by DCA and cortisone, some lesser activity from Compound S, progesterone, and testosterone and none from dihydro- and tetrahydro-cortisone compounds A and L, and ACTH (Karnofsky, unpublished, data).

The remainder of the report consists of the studies of the action of steroids against transplantable tumours in animals. The technique has consisted of daily subcutaneous injections for one week of saline suspensions, starting one day after subcutaneous implantation of the tumour implant. The tumours are measured in two diameters by calipers one week later and at subsequent weekly intervals. The results obtained with cortisone against a variety of tumours is included in Table IV. The maximum tolerated dose has been 37·5 mg./k/day (0·75 mg./mouse/day) and it has shown delayed complicated metabolic side effects. The strongest inhibitions have been obtained with the lymphosarcomas and osteogenic sarcomas. Mammary adenocarcinoma EO 771 has been inhibited moderately, while sarcoma 180 has not been affected

Table III

STEROIDS INACTIVE AS TESTED AGAINST THE CHICK EMBRYO

Compound	Max. dose level tested, mg/egg
Compounds with an 11 oxygen function and a saturated A and B ring	
11-keto-pregnanolone acetate	− 5·0
3, 11, 20-triketo-4, 21-diacetoxy,17-OH pregnane	− 5·0
Dihydro-cortisone acetate	−10·0
Compounds without an 11 oxygen function and a saturated A and B ring	
Allo-pregnanolone	−10·0
Allo-pregnanediol, 3β, 17α-one-20-acetate 3 (Comp. L)	− 5·0
Pregnane-3α, 12α, 21-triol-2p-one, 21 acetate	− 5·0
A compound without an 11 oxygen function and an unsaturation at C_1	
Δ^1-17 hydroxyprogesterone	− 5·0
Compounds without an 11 oxygen function and no carbonyl group at C_{20}	
Δ^4-pregnene-triol one	− 8·0
3-keto-Δ^4-pregnane-17β-20, 21 triol	−10·0
[illegible]	− 7·5
[illegible]	− 5·0
[illegible]	− 5·0
[illegible]	− 5·0
[illegible]	− 5·0
A compound without an 11 oxygen function and unsaturated at C_{16}	
$\Delta^{5,16}$-pregnadiene-3β-ol-20-one acetate	− 5·0

To sum up, certain steroids have been found to inhibit chick embryo development at the following levels—

·02 mg.	Compound F
0·2–0·5 mg.	Corticosterone
	Kendall's Compound A
	Cortisone
	11-ketoprogesterone
	11-keto-17-hydroxy-progesterone
	DCA

aspects of these compounds are listed in Table V. Table VI presents similar details for a group of steroids which have not inhibited the lymphosarcoma even though most of them have been tested at 5–10 times the maximum dose level for corti-

EFFECT OF CORTISONE ON OSTEOGENIC SARCOMA IN MICE

CORTISONE: 7 14 21 28

CONTROLS: 7 14 21 28 days

1 2 3 4 5 6 7 8 9 10

1cm

sone. Although the inactive compounds in Table VI show a number of variations in structure, all of them lack an 11-oxygen function. So far, it appears that the active steroids require an 11-oxygen function, and a 3-keto group with the Δ^4 conjugated unsaturation. The 17-hydroxyl group is not essential but appears to increase the effectiveness. A 20-keto

Table IV

EFFECT OF CORTISONE ON VARIOUS TUMOURS IN MICE

Dose mg/kg/ day	*Sar* 180	*Sar* T241	*Sar M 4* 337	*Car.* 1025	*EO* 771	*H-P Mel*	*WOS*	*ROS*	*PLS*	*MLS*
37 5	−	+	−	±	+		++	++	++	++
25·0								++	++	++
12 5								±	+	++

− indicates no effect, ± indicates slight inhibition, + indicates moderate inhibition; ++ indicates marked inhibition

EO771	=	Mammary Adenocarcinoma EO771
H-P mel	=	Harding-Passey Melanoma
WOS	=	Wagner Osteogenic Sarcoma
ROS	=	Ridgway Osteogenic Sarcoma
PLS	=	Patterson Lymphosarcoma
MLS	=	Mecca Lymphosarcoma

at the maximum tolerated dose. The degrees of inhibition of the lymphosarcomas and osteogenic sarcomas are illustrated in Figs. 7 and 8. In the case of the lymphosarcomas an increase in survival time is also noted. Thus far, the histological studies of the inhibited tumours have not revealed more than a mixture of viable tumour cells and those with degenerating nuclei.

The results with cortisone encouraged the study of numerous steroids in the spectrum of tumours with emphasis upon the lymphosarcomas and osteogenic sarcomas. These studies have offered some information on the relationship of steroid structure to inhibition of the lymphosarcoma (Stock, 1950; Sugiura *et al.*, 1950). Compound F is nearly as active as cortisone, whereas two to three times as much compound A is required for comparable activity. 21-desoxycortisone has a very slight activity, probably no more than one-twentieth to one-fiftieth that of cortisone. Dihydro- and tetrahydrocortisone have shown no activity. The structural

Table V

STRUCTURAL ASPECTS OF STEROIDS TESTED AGAINST MOUSE LYMPHOSARCOMA

Compound	*Dose mg/kg/ day*	*Activity*	*11-Keto*	*11-OH*	*Δ^{4}-3-Keto*	*20-Keto*	*3-OH*	*17-OH*	*21-OH*
Cortisone	37·5	+	+	−	+	+	−	+	+
Compound F	37·5	+	−	+	+	+	−	+	+
Compound A (17-desoxy cortisone)	125	+	+	−	+	+	−	−	+
21-desoxycortisone	375	− to ±	+	−	+	+	−	+	−
Dihydrocortisone	375	−	+	−	−	+	−	+	+
11-keto pregnanolone	150	−	+	−	−	+	+	−	−
Compound S (11-desoxycortisone)	450	−	−	−	+	+	−	+	+
Desoxycorticosterone (11, 17-desoxycortisone)	375	−	−	−	+	+	−	−	+

Table VI

STRUCTURAL ASPECTS OF STEROIDS INACTIVE AGAINST MOUSE LYMPHOSARCOMA

Compound	*Dose mg/kg/ day*	*11-oxy*	*Δ^{4}-3-keto*	*20-keto*	*3-OH*	*17-OH*	*20-OH*	*21-OH*
Testosterone	500	−	+	−	−	+	−	−
Progesterone	300	−	+	+	−	−	−	−
Δ^{5}-pregnenolone	50	−	−	+	+	−	−	−
$\Delta^{5,16}$-pregnenolone	187·5	−	−	+	+	−	−	−
Desoxycorticosterone	375	−	+	+	−	−	−	+
21-OH-pregnenolone	375	−	+	+	−	−	−	+
17α OH-progesterone (Δ^{4})	250	−	+	+	−	+	−	−
17α OH-progesterone (Δ^{1})	250	−	+ Δ^{1}	+	−	+	−	−
17β OH-progesterone	150	−	+	+	−	+	−	−
17α OH-allopregnanolone (Cmpd L)	200	−	−	+	+	+	−	−
17α, 21-OH-progesterone (Cmpd. S)	375	−	+	+	−	+	−	+
17α-triolone	300	−	+	−	−	+	+	+
17β-triolone	250	−	+	−	−	+	+	+

group has been present in the active steroids, but there has not been an opportunity to test the 20-hydroxy compounds. The 21-hydroxyl group is quantitatively very important, if its presence is not essential.

EFFECT OF CORTISONE
ON LYMPHOSARCOMA IN MICE

CORTISONE 7 14 21 28 CONTROLS 7 14 21days

1cm.

Our studies upon the anti-tumour effects have not included a survey of the organ changes. Dr. Woolley has been investigating these effects and has reported that the compounds with an anti-tumour activity in mice depressed the weight

DISCUSSION

SHOPPEE. I was rather surprised to see that in the egg yolk sac experiments Kendall's Compound F showed about 10 times the activity

secondary di-alcohols suggests that they are not metabolized very rapidly.

HADDOW: How reproducible are these different runs?

STOCK. They have been very satisfactorily reproducible, but I don't

process for the human organism. Birds are very different from mammals, and one has to be extremely careful about jumping to conclusions about humans from work on birds. Maybe Dr. Stock can say a few words about the actions of Compounds E, F and A.

STOCK: In our initial experiments with Compound A, it was found active even though somewhat larger amounts were required, and we had the impression that the metabolic side effects were not as bad.

Dr. Karnofsky has been interested to see whether the results in the egg would be carried over to the young chick. He injects the chicks with various doses, usually of the order of 1 mg. per day per chick,

Dr. Burchenal has tested a few of these materials in his mouse leukæmia. He hasn't had the opportunity to test a lot of them because the technique he has used required larger amounts, which were not available.

of the spleen to one-third normal, and the weight of the thymus to one-tenth normal; also they decreased the size of the mesenteric lymph nodes, of the adrenal cortex and of the pituitary. Dr. Money has made similar observations in rats.

Summary

It has been found that cortisone inhibits the development of certain transplantable mouse tumours, osteogenic ones and lymphosarcomas, whereas other tumours are less affected or not at all. Differences in the degree of inhibition of the lymphosarcomas by various steroids suggest relationships of structure to activity. It is believed that the chick embryo and the mouse tumour studies offer methods of screening steroids for biological activity which are both rapid and economical of material. These may be useful for selecting steroids for clinical trial in cancer.

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[illegible] at National Center Sym- [illegible] 1950.
[illegible] C. P. (1950).

a year.

laboratory which is interested in the production of useful steroids by fungi. They are interested in this test as a possible method of picking up activity.

GROSS: Did you try ACTH too in this test?

demonstrating any possible activity.

tu

STOCK: Yes. For one of our controls we have had animals on a starvation diet where there has been a comparable loss in weight without tumour inhibition. There isn't any question that there is some toxicity. In a few of the experiments nearly all the animals died at the end of the second week from delayed toxicity.

BEGG: I'm a bit uneasy about using this egg embryo test as a screening method. For example, I believe Dr. Karnofsky showed some activity for Compound S in eggs, yet none at all in the tumours.

STOCK: I certainly wouldn't propose this as the ultimate test, but we're not too worried about a few false positives, just as long as there aren't too many of them. It was not expected that the egg test and the tumour test would necessarily yield parallel results.

BEGG: I wondered if we are not seeing examples of the fact that

Stock's studies on pre-natal chicks, we have used the œstrogens as a magnifying glass to bring out differences in tissue growth effects on the genital tract, and, as I showed you yesterday, have found that progesterone has a marked inhibitory effect on œstrogen-induced tissue growth in the genita ... We have screened a considerable numb ... and there is a very ... embryo as opposed

same. It may well be that some of them are effective by conversion

rious

t be

combination of several tests

SHOPPEE: Have you tried to correlate your different degrees of activity, not so much with the actual functional groups present, but simply with the number of oxygen atoms, as an indication of solubility in tissue fluids?

STOCK: We've thought about the possible influence of solubility but there seems to be no correlation.

FOLLEY: *I think that you will find that anhydrohydroxy progesterone* (pregneninolone or ethinyl testosterone), although it contains two oxygen atoms, is one of the most insoluble substances yet tested by the pellet implantation technique. You can't detect any loss in weight in a year.

mixtures. Only a small amount of material is needed. We know one laboratory which is interested in the production of useful steroids by fungi. They are interested in this test as a possible method of picking up activity.

GROSS: Did you try ACTH too in this test?

STOCK: I don't think it's been properly tried out. It hasn't shown much activity. In the animal tests we didn't give it quite frequently enough. We didn't have the opportunity of injecting "around the clock," and I understand from Dr. Woolley that that's essential for demonstrating any possible activity.

THE ANTI-ANDROGENIC CONTROL OF HUMAN CANCER

CHARLES HUGGINS

By anti-androgenic control is meant modification of the internal environment by orchiectomy or administration of œstrogenic compounds.

Anti-androgenic measures are of certain effectiveness in two human tumours: (1) cancer of the prostate; (2) cancer of the male breast. It is likely that this small list will be extended in the future.

As chemotherapeutic agents the sex hormones have one great advantage over all of the other medicines used in the treatment of cancer in that they are not toxic and if they do no good they wreak no great or immediate harm to the patient with cancer. In the treatment of cancer by sex hormones, the aim is to increase the resistance of the patient to his disease.

I shall talk mostly about prostatic cancer because perhaps more is known about this tumour than about any other. The development of the treatment of this lesion by medicines was not empirical; it was deduced from a series of laboratory observations which were carried out, I am afraid, very slowly and at a low intellectual level. The results by good fortune happened to have a sharp focus because the prostate glands are relatively simple, being acted upon, so far as is known, by only two types of hormones, the androgenic and œstrogenic compounds.

Actually, anti-androgenic control of some cancers was devised as a by-product of experiments on dogs in which a quantitative assay technique was instituted. The prostatic secretion is the end product of the male genital hormone complex in which the anterior pituitary, certain hormone

producing cells of the adrenal cortex and testis, and the prostate itself are involved. The prostatic secretion was collected at frequent intervals for rather long periods of time and the effects of substances with endocrine activity on the output of the gland were investigated. In brief, orchiectomy and œstrogen administration caused a decrease of secretion. But androgenic substances initiated, and maintained an augmented prostatic secretion.

It has been said that in any successful experiment there is an element of luck. The good fortune in this work (I do not assert that it was strikingly successful) was the selection of the dog as the subject; this species is the only one other than man to develop spontaneously tumours of the prostate. It was inevitable that sooner or later old dogs with these lesions should be encountered. As a matter of fact, after a short period of vexation because the lesion was interfering with a physiological study, a search was made specially for dogs with this condition.

It was observed that anti-androgenic measures caused a rapid regression of this canine neoplasm whilst androgen increased its size. It was obvious that these effects must be investigated with respect to the malignant and the benign tumours of man, and distinct effects were observed in both conditions, but were more obvious in prostatic cancer.

Now, in science the method of proof is often of greater interest than that which is proven. The effects on disseminated cancer of the prostate were proven by chemical means, namely through a study of the acid and alkaline phosphatases of serum. Acid phosphatase, a component occurring in human prostatic epithelium in rich concentration, had been found, by Gutman and Gutman in 1938, to be increased in the blood serums of certain patients with disseminated prostatic cancer. Now prostatic cancer frequently metastasizes to the bones, in which tissue it flourishes, evoking an overgrowth of the osteoblasts, and Kay had shown in 1929 that when the osteoblasts are stimulated the alkaline phosphatase of serum increased. We could then make a synthesis of these

facts (1941) and study the course of the disease by determining the levels of these enzymes at frequent intervals; of course, acid phosphatase indicated the activity of the malignant cells, while the quantity of alkaline phosphatase indicated the reactivity of non-malignant tissue which was the host of the cancer. In brief, the administration of testosterone increased the activity of the tumour, and anti-androgenic measures caused a decrease of acid phosphatase toward or to normal. Also when the lesion was brought under control there was at first a rise of alkaline phosphatase, indicating a stimulation of osteoblasts (frequently due to a filling in of the bone lesions in the healing process) with a return to normal after some weeks.

The results of anti-androgenic control are of interest from several standpoints. The effect of control is often catastrophic to the tumour and of course beneficial to the patient. There occur an increased appetite and intake of food, and relief of pain. Protein synthesis begins at a rapid rate. The vicious circular chain is broken and the invalid is relieved. Now the striking feature about all of this is its speed; improvement may be detected by chemical means within 24 hours.

The minimal effective dosage of stilbœstrol needed to bring about control has been determined; it is between 0·25 and 0·3 mg. a day of diethyl stilbœstrol administered intramuscularly, thus establishing the fact that control lies within the physiological limits. Diethyl stilbœstrol happened to be the first non-radioactive substance which was found effective against widespread cancer in man.

Statistically, about 95 per cent of patients with prostatic cancer obtain some relief, which is usually considerable. However, a relapse occurs in about 75 per cent of the patients after 6-18 months and these we designate as the failure cases. On the brighter side 20 per cent of patients in our first series are alive and in good health after 10 years, without evidence of disease by chemical or clinical methods. In these people the control certainly has been fairly extensive and moderately long.

In the failure cases certain factors may be discerned. (1) In some of those who relapse there is production of androgen in extra-genital depots which have been identified as the suprarenal glands. (2) Certain of the cancer cells acquire or retain the property of androgen-independence. We conceive of control as the withdrawal, in effect, of androgen from certain cells which require it as an essential nutritive element. Some of the prostatic cancer cells find ways of maintaining themselves in the complete absence of androgen so that escape from control is inevitable.

It should be mentioned that the results from orchiectomy are clinically superior to those obtained from œstrogen administration. In fact, the two methods of androgenic control are in certain ways fundamentally different. After orchiectomy the hypophysis is stimulated and there is much to suggest that the consequent increased adrenal activity is beneficial. Oestrogen in man, on the contrary, depresses the pituitary and this seems to be less beneficial to the patient. Excision of the gonads is the only method known to increase the activity of the pituitary gland for long periods of time.

Since these studies were published, Farrow and Adair have shown that orchiectomy often causes massive regression of the rather uncommon condition of cancer of the male breast. These fine observations of the Memorial Hospital workers have been confirmed in many quarters.

In summary, it may be stated that modification of the internal environment of the host is capable of effecting long continued control of certain widespread cancers of human beings.

DISCUSSION

FOLLEY: You showed a slide of the weight of the dog prostate in which the dog was starved and given testosterone and the prostate grew. You said that you thought it was probably due to waste tissue coming from the catabolism of the body. Have you ever done that experiment in an adrenalectomized dog? As you know, adrenalectomy cuts down tissue catabolism.

HUGGINS: No, we haven't done that. This is a rather widely diversified activity of tissues of the genital tract. The salmon, for example,

in swimming up the river exhausts his proteins but turns out a magni-

sections look much the same?

HUGGINS: I don't go on sections.

BURROWS: Does the innocent prostate react towards œstrogen in the same way as the malignant ones?

HUGGINS: Yes, it does. It takes quite a lot longer to react. It takes, say, 90 days, whereas a malignant prostate will react in one or two

ptoms because

still maintain

oped following

studied them from the standpoint of acid phosphatase content. I'm not entirely persuaded that these are not metastatic nodules to the breast whose soil has been prepared by œstrogen.

BEGG: This might be a manifestation of multiple tumours. Someone once showed that if you remove the breast cancers from mice who

and gets a breast cancer that the stilbœstrol necessarily caused the cancer.

HERTZ: It is well established that those who have one primary carcinoma have a greater susceptibility to a second primary than the rest of the population.

HORNING: Do prostatic carcinomas in dogs ever form metastases in the bones?

HUGGINS: They are mostly benign tumours. To the best of my knowledge, spontaneous carcinoma has never been observed in the living animal. Histologically we find that the incidence of carcinoma in these dogs is about 2 per cent, but they have not metastasized.

ADMINISTRATION OF MASSIVE DOSAGE OF ŒSTROGEN TO BREAST AND PROSTATIC CANCER PATIENTS; BLOOD LEVELS ATTAINED

ROY HERTZ, JOHN PAUL YOUNG and W. W. TULLNER

NUMEROUS clinical reports have established the definite though limited effectiveness of œstrogen therapy in selected cases of prostatic and breast cancer (Huggins, Stevens and Hodges, 1941; Nathanson, 1947).

The transient character of the therapeutic effect of œstrogens in prostatic cancer has been noted by many observers. In breast cancer, both the relative infrequency and the temporary character of œstrogen-induced regressions constitute definite limitations to the practical usefulness of this form of hormone therapy. Nevertheless, the decisive clinical effects obtained with œstrogens in both prostatic and breast cancer command further study aimed at fuller knowledge of the optimum conditions for obtaining such favourable effects.

We have considered the factor of dosage as of paramount importance. By dosage, we do not simply mean the total amount of drug given to the patient, but rather refer to the amount given in relationship to the maintenance of an effective blood level for a stated period of time. In antibiotic therapy, these phases of the dosage problem have been greatly clarified, and the role of massive dosage in the management of such otherwise non-responsive conditions as subacute bacterial endocarditis has been defined. The present report represents a first step in our continuing attempt to determine whether similar principles of dosage and blood level are applicable to the problems of increasing the effectiveness of œstrogen therapy in cancer patients. Moreover, our studies afford data

concerning the tolerance to, and metabolic fate of massive œstrogen dosage.

The diagnosis in all of our cases was initially established by biopsy. Only those patients who were not amenable to any of the established methods of clinical management were selected for these studies. The progress of these patients was followed by constant clinical observation in a research ward. Evidence of alteration in their lesions was recorded by initial and periodic photographs under fixed conditions of illumination and magnification. Toxic reactions to administered œstrogens were continuously assessed by appropriate biochemical studies including such determinations as cephalin flocculation, bromsulphalein retention, serum bilirubin, prothrombin time, eosinophil response to adrenaline, serum calcium and phosphorus, acid and alkaline phosphatase, and frequent urinary and hæmatological analyses. Radiological study of the progress of osseous lesions was carried out when indicated.

We have employed in the major portion of these studies an injectable concentrate of naturally occurring conjugated œstrogens prepared from pregnant mare's urine.* In addition, a more limited number of studies has been done with orally administered ethinyl œstradiol.†

We have recorded previously (Hertz, Tullner, Westfall, Morrow and Emge, 1949) our preliminary studies on a series of breast and prostatic cancer patients. Our current body of data represents observations in fifteen prostatic cancer patients and thirty-six breast cancer patients. Our experience to date includes the administration of 168 intravenous infusions of from 100 mg. to 1,000 mg. equivalents of œstrone sulphate, and 312 subcutaneous infusions of 50–1,000 mg. equivalents of œstrone sulphate.

*The preparation used was "Premarin" (injectable) kindly supplied by Ayerst McKenna & Harrison Ltd., through the courtesy of Drs. G. H. C. McKeown and E. C. Reifenstein, Jr.

†Ethinyl œstradiol was obtained through the Committee on Therapeutic Trials of the A.M.A. and from Ciba & Co., through the courtesy of Dr. E.

We have determined the œstrogen blood levels at varied intervals following the administration of a wide range of dosages given subcutaneously, intravenously, and orally. These blood levels are determined by assaying the biological effect of the patient's serum on the weight of the uterus of ovariectomized infantile rats, according to the method of Lauson, Heller, Golden and Sevringhaus (1939). Determinations of blood levels of œstrogens have been made at varying intervals following 87 intravenous infusions, 40 subcutaneous infusions and 21 oral treatments. All assay values are expressed in microgram equivalents of œstrone sulphate per ml. of the patient's serum.

As an example of our quantitative studies of the relationship of dose, injection route, and blood level at varying intervals following treatment, we have selected for presentation here our pooled data obtained from patients given a test dose of 300 mg. of œstrogen. Fig. 1 shows the blood level curve after intravenous infusion of this dose in about 30 minutes in a total volume of 300 ml. A mean blood level of 35 μg. per ml. is obtained and this rapidly falls away with only a very occasional patient showing a significant blood level at the end of 24 hours. The bars indicate the range of variability of the determinations and the number of parentheses indicates the number of determinations made on a given point.

We also studied the effect of giving 300 mg. subcutaneously in 300 ml. volume over about one hour's time. The blood levels attained are of about the same order and these blood levels are maintained for a somewhat longer period of time. Nevertheless, the 24 hour blood level, although somewhat more variable, is only slightly higher than that seen after intravenous infusion.

Oral administration of doses of both crystalline ethinyl œstradiol and of conjugated œstrogens, which approach the limit of gastric tolerance, have afforded much lower blood levels. For example, the highest blood level observed following orally administered conjugated œstrogens was 8 μg. per ml. and this resulted from the ingestion of a total of 3·6 g.

in 29 days. Similarly, after the ingestion of 10 mg. per day for 19 days of ethinyl œstradiol a blood level of only 5 μg. per ml. was observed.

Thus, our data afford useful information regarding the relative lack of toxicity of dosages previously considered to

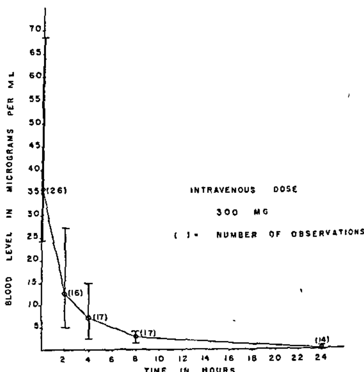


FIG 1. Blood level of œstrogen after intravenous infusion of 300 mg œstrogen in 300 ml. solution over 30 minutes. Pooled data.

exceed the limits of tolerance. In addition, the blood level data indicate the practicability of procuring by parenteral administration blood levels which exceed any that have been obtained by oral administration so far. However, the rapidity with which even the highest blood levels fall away suggested that almost continuous œstrogen administration would be required to maintain a consistently high blood level.

Accordingly, we have made some preliminary studies, employing intravenously placed plastic catheters, of continuous intravenous infusion of large doses of injectable œstrogen in seven patients with advanced cancer of the prostate. This limited experience has indicated that such prolonged infusions may prove a practicable method of maintaining a higher blood level for a longer period of time than is otherwise possible. Fig. 2 shows the record of a twelve hour continuous infusion of a total of 2,330 mg. of œstrogen in the case of a debilitated

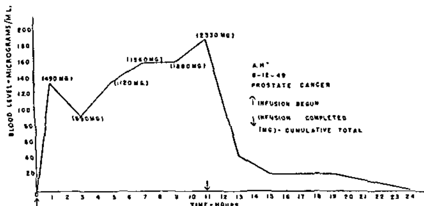


FIG. 2 Blood level of œstrogen in prostatic cancer patient during 12-hour infusion of 2,330 mg. of œstrogen.

prostatic patient. The amount infused at various time intervals is indicated in parentheses and the blood level of œstrogen is graphed against time. Fig. 3 represents our most prolonged infusion to date; it lasted for 72 hours. This involved the administration of 13·3 g. of œstrogen and the almost constant maintenance of a blood level of 400 μg. per ml.

Toxic side effects of such intensive œstrogen administration have proved less severe and less frequent than had been anticipated. Œdema of significant degree was seen in our entire experience in only three patients and this subsided

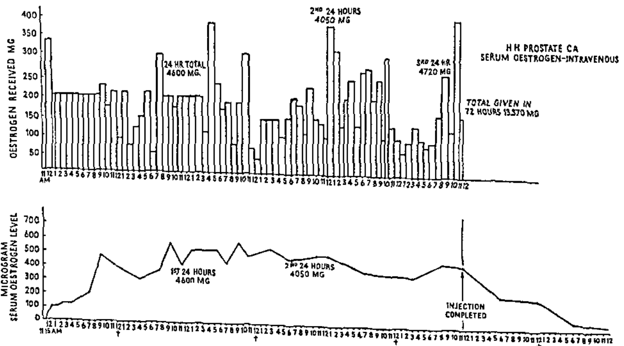


FIG. 3. Blood level of oestrogen in prostatic cancer patient during 72-hour infusion of 13·3 g. oestrogen.

upon interruption of the œstrogen treatment for three to four days and with the use of a salt-free diet. Nausea of varying degree was a recurring complaint of almost all patients in whom a high blood level was maintained for any length of time. In the prostatic cancer patients receiving the more prolonged intravenous infusions, vomiting occurred in all seven but could be controlled in those patients given a liquid diet during the course of the infusion.

In addition, an aged debilitated woman suffering from widely metastasizing breast cancer and severe hypertension died about five minutes after the intravenous infusion of only 400 mg. of œstrogen. The clinical features suggested a cerebrovascular accident. However, a careful autopsy, including complete examination of the brain, showed no apparent basis for her death other than extensive carcinomatosis and hypertensive cardiovascular disease. Also, one of the prostatic cancer patients, 62 years of age, who had been given 40 hours continuous intravenous infusion of injectable œstrogen abruptly presented a picture of peripheral vascular collapse necessitating discontinuation of the infusion. His pulse became rapid and thready and his blood pressure was unobtainable. His abdomen was noted to be distended and rigid and epigastric tenderness was elicited. Since he had had in the past a perforated peptic ulcer requiring surgical intervention, it was considered that he then was again suffering from perforation. During the ensuing two days, he responded quite well to transfusions and other supportive measures and a blood urea nitrogen of 44 mg. per cent was reported. On the third day, he became comatose and irrational and died. Autopsy revealed an intact gastro-intestinal tract. The kidneys showed a diffuse parenchymatous swelling associated with lower urinary tract obstruction due to extensive carcinomatous invasion. It was considered that death had occurred from uræmia.

Hence, despite careful autopsy examination in these two cases, it remains difficult to determine the role of the œstrogen as a toxic factor. However, in view of the lack of major

toxicity in numerous patients much more intensively treated than these two, it is reasonable to consider that the fatal course in these two patients can be more readily attributed to other phases of their advanced disease-state.

Laboratory studies referred to above have indicated no evidence of hepatic, renal or hæmatological damage in any of our cases.

Our data so far are admittedly of more pharmacological than therapeutic interest. We have seen significant therapeutic effect from massive œstrogen dosage in five prostatic cancer patients and in five breast cancer patients. In the prostatic cancer patients these favourable effects included: (1) a rapid reduction in size of the palpable prostatic mass; (2) relief of severe bone pain with an associated abrupt drop in serum acid phosphatase levels; (3) general clinical improvement as regards weight, appetite and feeling of well being. In the breast cancer cases, these effects have included: (1) prompt and marked regression in the visible breast lesion; (2) marked suppression of pleural effusion; (3) decisive relief of pain from bone metastases; (4) reduction in size of metastatic lymph nodes; and (5) general clinical rehabilitation of the patient.

Fig. 4 shows the treatment record over a period of 49 days of E.B., a 75 year old white female who had received no prior treatment for her breast cancer. One thousand milligrams of œstrogen subcutaneously were given almost daily throughout the entire period. Fig. 5 shows the initial appearance of the breast lesion in contrast to its appearance when treatment was discontinued seven weeks later. The gross regression which was accompanied by a marked rehabilitation of the patient is apparent. Fig. 6 shows the course of this lesion in greater detail at about two week intervals during therapy. Such rapid and decisive clinical effects as this have stimulated our interest in further evaluating this intensive form of hormone therapy.

In conclusion it should be emphasized that this report is offered to indicate only some initial progress toward



A



B

Fig. 5. Patient of Fig 4 before treatment (A) and after seven weeks treatment (B).

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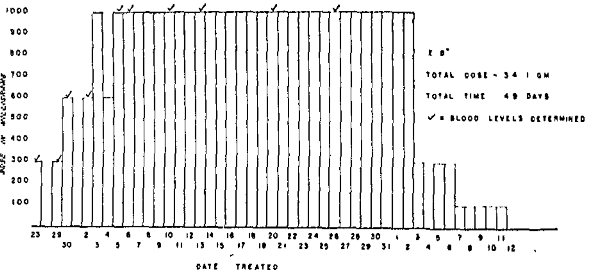
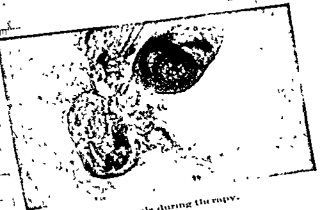


FIG. 4. Œstrogen treatment record of breast cancer patient, given 34.1 g. œstrogen over 40 days.



4 and 5 at two-week intervals during therapy.

better.

I'm not at all persuaded that tremendous doses of these things are what is needed. I think, from my limited experience, that minute

HERTZ: In the two patients who died in the course of these studies the adrenal was not unusually large; they had extensive carcinomatosis. And we have also followed very carefully the eosinophil reactivity to an administered dose of adrenaline of the patients during the course

tremendous amounts of chromogenic material.

DOBRINER: There are a number of reports that in mice, rats and rabbits the adrenals increase in size after treatment with œstrogens, but this may not always involve an increase in adrenal function.

allow a cancer cell to adjust to the œstrogen by teasing it with small dosages we may be able to suppress it more effectively

HADDOW: Our experience at the London Cancer Hospital in both

establishing a quantitative base line in œstrogen therapy of cancer so that more effective forms of hormone therapy may ultimately be evolved. Our data are to be interpreted as indicating: (*a*) the feasibility of massive parenteral œstrogen administration; (*b*) the quantitative limits of the blood levels attained; and (*c*) sufficient clinical therapeutic effect to justify further exploration of massive œstrogen therapy under controlled conditions of clinical study.

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DISCUSSION

HERTZ: I failed to mention that we have used progesterone effectively and have seen no toxic side effects, except in one patient who was sensitive to the oil vehicle and showed an allergic reaction, so that we felt that progesterone was sufficiently inert to allow us to go up to very high dosages. Other steroids will have to be approached much more cautiously.

STOCK: Although progesterone showed marked activity in our tests, I would not have expected it to be effective necessarily in clinical trials, because we would have anticipated that there would be false positives. On the other hand, we may be missing activities in some [illegible] and we [illegible] we [illegible] ave [illegible] lear [illegible] hat the clearing was of renal origin?

HERTZ: Yes. We haven't completed our studies, but we know that 30–50 per cent of the administered œstrogen given in such massive dosage is found in the urine in the next 26–36 hours. However, even when such an enormous dosage is given, we still can't account for it all, and perhaps there is an additional inactivating mechanism involving the inactivating capacity of other tissues. There may also be loss from the bowel, and we are intending to study that.

HUGGINS: At one time we thought it would be a good idea to synthesize stilbœstrol phosphate because it is a water-soluble ester. It

one because of the small amount of sulphatase, other esters might be better.

I'm not at all persuaded that tremendous doses of these things are what is needed. I think, from my limited experience, that minute

HERTZ. In the two patients who died in the course of these studies the adrenal was not unusually large; they had extensive carcinomatosis. And we have also followed very carefully the eosinophil reactivity to

initial eosinophil level, which would be low if the adrenal were activated. The biochemical studies on the urine are limited by the presence of

Hertz's results The only similar investigation I know of is one carried out by Dodds. It was not published, although I think there is a reference to it in the annual reports of the British Empire Cancer

Campaign two or three years ago. He administered amounts of the order of grams of one or more water-soluble derivatives. Perhaps Boyland could remember which ones?

Dodds found stilbœstrol sulphate and hexœstrol

there's

jection

a con-

would

have to convert it into a water-soluble derivative before excretion, and the blood concentration would be maintained. In general, as *Albert has stressed, increasing the solubility of a substance very* frequently reduces the biological activity.

HERTZ: *We have studied the blood levels in patients who were carry-*

of both stilbœstrol and steroid types of

Our working hypothesis

we can attain a blood

get a therapeutic effect

whether or not it is biologically active, the vaginal smears in these patients show very definite cornification.

BEGG: Did you show progesterone inhibition of œstrogen-induced tissue growth in the mammal?

HERTZ: In the mammal in moderately low dosage progesterone is *complementary to the growth-stimulating action of œstrogen*, in terms of weight increment in the genital tract. However in some of our studies, when we used large dosages, 3–4 mg. a day, we found the same

rogesterone did you notice any

Trentin has recently observed

nice that have received, over a

f progesterone a day. It is a rather significant increase in weight, more than you can get in these animals with testosterone. He hasn't had it analysed for fat or protein yet.

I think that Dr. Folley mentioned that the mammary glands,

grew disproportionately, and there is

the monkey's

fect for a long

far. I think it

ies or organ to organ.

Or from benign to malignant?

ht very closely and have

weight due to improve-

ve have been concerned

ie as seen in pregnancy

reflected to some extent

For that reason we

studied the histidine excretion in these patients, and found that progesterone, even in these large doses, will not give the histidinuria, whereas cortisone will. Pregnanediol excretion, as done by Sommerville and Marrian's method, has been within the expected range of normal recovery, indicating that we're not dealing with any great abnormality in steroid metabolism in these patients.

BURCHENAL: I would like to support Dr. Hertz's theory about the value of large dosage. We've certainly seen that with cortisone in acute leukæmia. A child given 50 mg. a day, which will work with some children, may go on for two months with no improvement at all; then you increase the dose to 100 mg. a day and even with that you get no improvement; but if you get it up to 200 mg. a day, you may see a rapid betterment of the general condition and a reversion of the bone marrow to normal.

GROSS: Can you observe the same kind of regression with testosterone in cervical cancer?

HERTZ: We have not studied the effect on cervical cancer.

DOBRINER: I think we have to correct our thinking about dosage. The amounts one used to call "veterinarian" are produced by humans. For therapeutic application, I think one has to see what an *adequate* amount is. It is possible that there is a maximum dosage, and an increase doesn't help. We have to pay much more attention to the blood level. One reason that Compound E, for instance, is given in four or eight doses per day is that one has to attain a certain blood level. The same thing may be true for the œstrogens and testosterone. I think that Dr. Hertz is on the right track when he uses such a high dose. If we could release the hormone steadily and imitate the action of the glands, I think our therapy would be very much more successful.

HADDOW: At the Cancer Hospital we had almost stopped considering any more the use of large amounts of hormones, but Dr. Hertz's paper makes us think about them again. I am particularly impressed by the dramatic regression with progesterone withdrawal

THE MODIFICATION OF TUMOUR-HOST RELATIONS BY STEROID HORMONES

R. W. BEGG

I. Systemic Effects of Tumours in Rats

SYSTEMIC effects of tumours are regarded as the changes produced in tissues of the host which are remote from the tumour, and in which no evidence of metastatic malignant cells is found. They may be anatomical, of a nature described by Parsons (1947), or of the type of biochemical change reviewed by Greenstein (1947).

It has been suggested on histological grounds that the clinical state of malignant cachexia is due to hypofunction of the adrenal (Dalton, 1944) and this view may be supported by certain clinical studies (Potor *et al.*, 1948; Reifenstein *et al.*, 1948; Young *et al.*, 1948). In view of the number of metabolic factors known to be influenced by the adrenal cortex (Thorn and Forsham, 1949), the possibility that some systemic effects could be explained by a primary action on the pituitary-adrenal system was investigated.

Liver catalase activity was chosen for the study as a well established systemic effect (Adams, 1950; Appleman, Skavinski and Stein, 1950; Dounce and Shanewise, 1950; Greenstein, Jenrette and White, 1941); adrenalectomy leads to a diminished activity of liver catalase (Begg and Reynolds, 1950). Hæmoglobin and liver catalase have a common prosthetic group (Theorell, 1947) and anæmia has been related to tumour growth (Taylor and Pollack, 1942) and to the adrenal cortex (White and Dougherty, 1945).

The enlarged adrenal, low in ascorbic acid and cholesterol, which has been described in the tumour-bearing animal (Savard, 1948; Haven, Bloor and Randall, 1949) would be compatible with exhaustive hypofunction of the adrenal

cortex (Sayers and Sayers, 1948), particularly in conjunction with the diminished liver glycogen deposition (Young, Kensler, Seki and Homburger, 1947) and lymph node hypertrophy (Homburger, 1948) which have been demonstrated in tumour-bearing mice.

It was decided to study these multiple systemic effects in a single animal at different stages of tumour growth, and the rat was chosen to provide the required amount of tissue. Thymus weight was followed as an example of lymph tissue, for it was assumed to react to experimental procedures in a manner similar to lymph nodes (White, 1947).

Young male Sprague-Dawley-Holtzman rats were maintained on a diet of Purina fox chow and tap water in a room maintained between 72 and 78°F. The rats were bred in the laboratory or supplied by the Holtzman Rat Company, the latter being adapted to the animal room for at least ten days before use. Bilateral grafting was done aseptically when the rats were approximately six weeks of age, a tumour suspension being used. The Walker 256 carcinoma was grafted either subcutaneously or intramuscularly, but only the latter method was used for the Jensen sarcoma. Normal male rats served as controls and were killed at the same time as tumour-bearing rats. Twenty hours before sacrifice the rats were placed in clean cages without food but with water supplied *ad libitum*. At this time hæmoglobin was determined on tail blood (Evelyn, 1936).

The rats were injected intraperitoneally with sodium pentobarbital in warm normal saline at a dose level of 5 mg. per 100 g. body weight. Under anæsthesia the abdomen was opened, the right adrenal transferred to formol-saline and the left placed in a dish of ice-cold normal saline. A second operator removed a piece of liver for glycogen determination (Good, Kramer and Somogyi, 1933). This was rinsed in ice-cold saline, blotted dry, weighed and introduced with minimum delay into a tube of hot 30 per cent potassium hydroxide. Meanwhile the left adrenal had been freed of adherent fat, blotted dry, sectioned with a razor blade and weighed on a

torsion balance while wrapped in cellophane. One piece was introduced into trichloracetic acid for estimation of ascorbic acid (Roe and Kuether, 1943). The other half was homogenized with a loose pestle in a tube containing acetone, and an equal amount of absolute alcohol added for the extraction and determination of cholesterol (Sperry, 1938) The remainder of the liver was ground and extracted for the estimation of liver catalase activity (Greenstein, 1942) and the thymus weighed on a torsion balance. After 48 hours fixation the adrenals were washed in running tap water for an hour, sectioned on a freezing microtome and stained with Sudan IV (Conn, 1946).

The body weights recorded are those prior to sacrifice and include both tumour and carcass weight. The tumours were measured in two diameters and are presented as the mean diameter of both tumours. Tumours were not weighed in all cases, but sufficient data are available to state that in the 20 mm. group they formed approximately 5 per cent of the body weight, in the 30 mm. group, 15 per cent and in the 40 mm. group 30 per cent of the body weight. With the subcutaneous grafts the tumours attained 10, 20, 30 and 40 mm. diameters in one, two, three, and four weeks respectively. The intramuscular grafts grew at a somewhat faster rate.

Values of P in the *t* test of Fisher are regarded as significant at the 0·05 level and highly significant at 0·01 (Snedecor, 1946).

Results

The Walker and Jensen tumours gave comparable systemic effects and the results obtained from rats bearing these tumours have been combined.

The mean body weights of the different groups are given in Table I. There is no significant difference between the mean weights of the groups, and the adrenal and thymus weights are thus regarded as subject to valid comparison between groups.

Thymus and adrenal weights are presented in Table II. There is a progressive increase in adrenal weight and a fall in

thymus weight after the tumours have attained a size of 20 mm.

The results of the estimation of hæmoglobin and liver catalase activity are tabulated in Table III. Hæmoglobin

Table I

BODY WEIGHT OF CONTROL AND TUMOUR-BEARING RATS

Tumour size	*Body weight (g)*
Control	170±4(7)
10 mm	160±7(16)
20 mm.	161±7(10)
30 mm.	173±11(10)
40 mm	182±12(10)

±Standard error of the mean.
Number of observations in brackets.

Table II

EFFECT OF TUMOURS ON ADRENAL AND THYMUS WEIGHT

Tumour size	*Adrenal weight (mg)*	*Thymus weight (mg)*
Control	15·4±0 8(7)	387±2 3(7)
10 mm	17·5±0 4(16)†	420±85(10)
20 mm.	18 8±0·3(10)*	324±45(12)
30 mm	21·3±2 0(10)†	288±86(5)
40 mm.	30 2±4·0(9)*	156±29(8)*

±Standard error of the mean.
Number of observations in brackets.
*P< 0 01 in *t* test
†P< 0 05 in *t* test

Table III

EFFECT OF TUMOURS ON HÆMOGLOBIN AND LIVER CATALASE ACTIVITY

Tumour size	*Hæmoglobin (g /100 ml)*	*Liver Catalase Activity ($K \times 10^4$ at 0 1 mg N/ml)*
Control	15 4±0 3(7)	1570±74(6)
10 mm.	16 2±0 8(16)	2079±132(16)*
20 mm.	11 8±1 2(10)†	1596±119(10)
30 mm.	10 7±1 1(10)*	1332±125(10)
40 mm.	8 6±0 5(10)*	753±81(10)*

±Standard error of the mean.
Number of observations in brackets
*P< 0 01 in *t* test
†P< 0 05 in *t* test.

falls progressively from the 20 mm. tumour to attain a pre-agonal value of approximately 50 per cent. The loss of liver catalase activity is of the same order. The increase in catalase activity in rats bearing small tumours has been reported (Greenstein *et al.*, 1941) and is significant in this series.

Table IV

EFFECT OF TUMOURS ON ADRENAL CHOLESTEROL AND ADRENAL ASCORBIC ACID

Tumour size	*Adrenal cholesterol (mg /100 mg)*	*Adrenal ascorbic acid (mg /100 mg)*
Control .	4·68 ± 0·52(7)	0·413 ± 0·012(7)
10 mm. . .	4·16 ± 0·38(16)	0·372 ± 0·012(15)†
20 mm. .	3·79 ± 0·52(10)	0·324 ± 0·017(10)*
30 mm. . . .	3·25 ± 0·45(9)	0·314 ± 0·022(9)*
40 mm. .	2·97 ± 0·44(10)†	0·238 ± 0·027(9)*

± Standard error of the mean
Number of observations in brackets
*$P < 0·01$ in *t* test.
†$P < 0·05$ in *t* test

In Table IV the effects on adrenal cholesterol and ascorbic acid have been recorded. The values for cholesterol and ascorbic acid fall to 60 per cent of the control at the 40 mm. stage.

Histological examination revealed a loss of sudanophilia from the adrenals of the tumour bearing rats. The loss of sudanophilia paralleled the loss of cholesterol from the adrenal.

Discussion

The Walker 256 carcinoma at an early stage of growth reduces the food intake of the host, and as the tumour increases in size the carcass loses weight (Mider, Tesluk and Morton, 1948). The suggestion has been made that this may be a factor in the loss of liver catalase (Dounce and Shanewise, 1950). Experiments have been reported which indicate that in the forcibly fed tumour-bearing rat no loss of carcass weight occurs, but systemic effects are present (Begg, 1950) Thus, failure to record food consumption in this study does not render the results invalid.

The present experiments are not in agreement with the statement that tumours forming 5 per cent of the body weight cause a diminution in catalase activity (Greenstein, 1947) but do agree with observations that half the liver catalase activity is lost from animals bearing tumours of 15 to 30 per cent of the body weight (Dounce and Shanewise, 1950). The catalase effect appears to be biphasic, as has been reported by Greenstein (Greenstein *et al.*, 1941) in rats but not noted by Adams (1950), who found an asymptotic relation between tumour weight and percentage of inhibition of catalase, in mice.

The earliest significant effect produced by a tumour in this investigation is the hypertrophy and loss of ascorbic acid from the adrenal at the 10 mm. stage, followed by anæmia at the 20 mm. stage. That the degree of anæmia is a consistent and reproducible finding in tumour-bearing rats is suggested by the fact that comparable groups of rats bearing the Walker carcinoma yielded hæmoglobin values of $9 \cdot 34 \pm 0 \cdot 49$, $8 \cdot 50 \pm 0 \cdot 40$ and $8 \cdot 03 \pm 0 \cdot 44$ g./100 ml. at intervals of six months.

It was suggested that diminished liver catalase activity and anæmia might be associated with hypofunction of the adrenal cortex in the tumour-bearing rat. Anæmia has been produced by adrenalectomy in mice (White and Dougherty, 1945), and it has been demonstrated that adrenalectomy lowers liver catalase activity in the rat (Begg and Reynolds, 1950). But the diminution in catalase activity in the rat from which the adrenals are removed is only half as great as in the tumour-bearing animal Thus it is unlikely that hypofunction of the adrenal cortex is a major factor in the production of anæmia and loss of liver catalase activity in the tumour-bearing rat.

The observed involution of the thymus in the tumour-bearing rat might be regarded as indicative of hyperfunction of the adrenal cortex (Selye, 1936) or inanition (Reinhardt, 1943). Extrapolation to another species should be done with caution, but thymus involution and lymph node hyperplasia have been observed in tumour-bearing mice (Homburger, 1948; Savard, 1948) and appear to be independent of the

pituitary (Savård and Homburger, 1949). This throws some doubt on the interpretation of the present data, and further studies should be done to investigate the response of lymph nodes as well as the thymus to the presence of a tumour in the rat, and the role of the adrenal (White and Dougherty, 1947).

The results obtained from the study of adrenal weight, adrenal ascorbic acid and cholesterol, and sudanophilia, might be interpreted as following the pattern of the stress reaction as exemplified by the Type III adrenal response of Sayers (Sayers and Sayers, 1948), on an extended time scale. This could be the result of an initial stimulation of the adrenal cortex being maintained and leading to eventual cortical exhaustion.

The progressive fall in adrenal cholesterol and ascorbic acid would suggest that the host was approaching the stage of adrenal cortical failure (Sayers and Sayers, 1948). In the absence of the determination of peripheral effects known to be influenced by the secretions of the adrenal cortex, these changes in the adrenal are difficult to interpret. The determination of liver glycogen in rats starved for sixteen hours gives such low results, and the data are so variable, that these experimental findings have not been presented. A plot of mean percentage change does indicate an initial increased storage of glycogen, with a diminution in the amount of liver glycogen in the terminal state. This problem should be studied with the use of intraperitoneal glucose (Young *et al.*, 1947).

It is probable that a state of adrenal cortical deficiency does occur in the tumour-bearing animal in the terminal stages of cancer, but further experimental substantiation is required. All of the systemic effects cannot be explained on the basis of this deficiency, and some other mechanism must be responsible for many factors known to be altered in the tumour-bearing host.

As the result of the present and related investigations it is necessary to explain how the presence of a tumour at a remote site brings about a stimulation of the adrenal cortex, a

diminution in the amount and activity of hæmoproteins and an involution of the thymus. Neither hormonal nor nutritional explanations seem to be adequate.

In his original studies on liver catalase in tumour-bearing rats Greenstein suggested that "the effect of the transplanted tumor on the liver catalase is elicited by a toxic substance produced in the tumor and carried by the blood to the liver" (Greenstein *et al.*, 1941). A recent abstract reports that such a substance can be extracted from a tumour (Greenfield and Meister, 1950). Adams (1950) favours the release of some substance from a tumour as an explanation of the diminished liver catalase activity of mice. A consideration of the systemic effects of tumours may lead to the revival of the concept of a cancer toxin and stimulate further research along this line.

A claim for specificity in relation to malignant tumours cannot be made for the observed systemic effects, and no explanation is available to account for these characteristic effects in tissues remote from a tumour. At the same time they are manifestations of the profound metabolic changes produced in the host, and as such it is felt that they play an important part in the fatal outcome of cancer.

II. Steroid Hormones and Tumour-Host Relations

It has been demonstrated that testosterone propionate in oil causes an increase in hæmoglobin and liver catalase activity in the normal rat, but that the administration of the steroid in large doses could not overcome the characteristic anæmia and loss of liver catalase activity in the tumour-bearing rat (Begg, 1949). It was of interest to see if these effects might be moderated by the administration of a more potent androgen.

The use of pellets of testosterone propionate provided another approach to the problem. Implanted in the subcutaneous tissues these would give a slow steady absorption of the hormone which might be of greater effect than daily injections in oil.

A pattern of the systemic effects of tumours has been described and evidence presented to suggest that rats in the terminal stage of cancer may be in a state of adrenal cortical insufficiency (Part I). If this reasoning were valid it might be anticipated that an exogenous supply of hormones of the adrenal cortex would modify the response of the host to the tumour.

Methods

A group of young male Sprague-Dawley-Holtzman rats bearing intramuscular grafts of the Walker 256 carcinoma in both thighs was used as test animals. On the ninth day of growth, when the tumours had attained a diameter of 10 mm., injections were begun or pellets implanted. The animals were maintained on Purina fox chow and tap water in a room controlled at 72–78°F.

Testosterone cyclopentylpropionate* (TCP) was given in two 5·0 mg. doses, one on the ninth and one on the fourteenth day of tumour growth. Lipo-Adrenal Cortex† (LAC) was administered in daily injections of 0·5 ml. increasing to 1·5 ml., different groups receiving 240, 360 and 420 rat units respectively as a total dose. Control animals were given appropriate amounts of cottonseed oil. Pellets of testosterone propionate‡ (TPP) were implanted over the scapular region, five pellets per rat, each placed at a discrete site. At autopsy the pellets were removed, dried and reweighed for the determination of total absorption.

Hæmoglobin was estimated on tail blood on the afternoon preceding sacrifice (on the twentieth day of tumour growth),

*Testosterone cyclopentylpropionate was provided by the Upjohn Company through the kindness of Dr. H F Hailman. The Upjohn Company report that this compound has a more potent and prolonged androgenic activity than testosterone propionate

…getable
milli-
pound

‡Pellets of testosterone propionate were provided by the courtesy of Dr B. L Frank of the Ciba Pharmaceutical Company (Canada). They weighed approximately 15 mg each and were known to give an absorption of about 0 2 mg per pellet per day

control rats being killed at the same time. The two androgen groups were given access to food and water till the end of the experiment, but the LAC group were starved for sixteen hours and given 400 mg. glucose intraperitoneally two hours before sacrifice.

Tissues were removed under nembutal anæsthesia and analysed by methods described in Part I.

Tumours were measured in two diameters of each tumour and are expressed as the mean of the four diameters. In these experiments the tumour weight corresponds to approximately 25 per cent of the body weight.

Results

Testosterone Cyclopentylpropionate

Table V indicates that the only significant difference between the control and treated groups is increased thymus atrophy in the rats given TCP. The mean adrenal weight is

Table V

EFFECT OF TESTOSTERONE CYCLOPENTYLPROPIONATE ON TUMOUR BEARING RATS

	Control	*T C P*	*P**
Body weight, g	245·1±7·3(10)	242·8±8·4(9)	>0·05
Tumour diameter, mm. . .	39·0±1·0(10)	39·1±1·3(11)	>0·05
Adrenal weight, mg	28·5±0·7(8)	26·5±1·2(11)	>0·05
Thymus weight, mg	103·4±20·7(8)	105·2±14·0(11)	<0·01
Hæmoglobin, g/100 ml	8·03±0·44(10)	7·67±0·72(11)	>0·05
Liver catalase, $K \times 10^4$	1964±142(10)	2053±213(11)	>0·05
Adrenal cholesterol, mg /100 mg. .	2·24±0·28(8)	1·73±0·20(11)	>0·05
Adrenal ascorbic acid mg /100 mg	0·315±0·029(8)	0·315±0·017(11)	>0·05

Number of observations in brackets
± Standard error of the mean
*Probability in *t* test <0·05 = significant
<0·01 = highly significant

smaller in the treated group, but the variation considerable; a similar response is noted in adrenal cholesterol. The failure to influence hæmoglobin and liver catalase activity is evident.

Testosterone Propionate Pellets

As indicated in Table VI there is no apparent effect on

Table VI

EFFECT OF TESTOSTERONE PROPIONATE PELLETS ON TUMOUR BEARING RATS

	Control	*Pellets*	*P**
Body weight, g.	245 1±7 3(10)	230·2±3 2(11)	>0 05
Tumour diameter, mm.	39 0±1 0(10)	38 6±1·0(11)	>0 05
Adrenal weight, mg.	28 5±0 7(8)	25 5±0·9(11)	<0 05
Thymus weight, mg.	193·4±20 7(8)	50 8±4 6(11)	<0 01
Hæmoglobin, g/100 ml.	8 03±0 44(10)	8 39±0 70(11)	>0 05
Liver catalase, K×10⁴	1964±142(10)	2309±118(11)	>0 05
Adrenal cholesterol, mg /100 mg.	2·24±0 28(8)	1 09±0·23(10)	<0·01
Adrenal ascorbic acid, mg /100 mg	0 315±0 029(7)	0 306±0 018(11)	>0 05

Number of observations in brackets.
±Standard error of the mean.
*Probability in *t* test. < 0 05 = significant.
< 0 01 = highly significant.

adrenal ascorbic acid but adrenal weight and cholesterol are both significantly lower in the treated group. The pellet treated rats showed a marked involution of the thymus but there was no significant effect on liver catalase activity or hæmoglobin.

The average absorption of testosterone propionate from the pellets was 0·99 mg. per rat per day.

Lipo-Adrenal Cortex

An examination of Table VII demonstrates that the injection of adrenal cortical extract produced significant changes only in thymus weight, hæmoglobin and body weight.

The mean weight of the two groups was the same at the beginning of the injections. It is difficult to interpret the decreased gain in body weight in the treated group as the animals were not tube fed, and the reduction in weight gain may be a reflection of a decreased food consumption. The

characteristic loss of sudanophilia in the adrenals of tumour-bearing rats was not so marked in the treated as in the control group.

Table VII

SPRAGUE-DAWLEY RATS BEARING WALKER 256 CARCINOMA

	Control	*Lipo-adrenal cortex*	*P**
Body weight, g.	323·4±7 2(11)	299·3±5 1(12)	<0·07
Tumour diameter, mm.	46·3±2 0(12)	45 8±1 2(12)	>0 05
Adrenal weight, mg.	29 4±1 6(11)	27 4±1 5(10)	>0 05
Thymus weight, mg	207 6±17·6(11)	88 0±6 0(10)	<0 01
Hæmoglobin, g/100 ml.	8 5±0 4(11)	10 4±0·5(12)	<0 01
Liver catalase, K×10⁴‡	933±94(11)	759±123(10)	>0·05
Adrenal cholesterol, mg /100 mg	2 64±0 30(11)	3 28±0 44(10)	>0 05
Adrenal ascorbic acid, mg /100 mg.	0 314±0 016(11)	0 291±0 015(10)	>0 05
Liver glycogen, mg /100 mg.†	0 44±0 05(11)	0 45±0 06(10)	>0 05

Number of observations in brackets.
± Standard error of the mean
*Probability in *t* test: < 0 05 = significant
< 0 01 = highly significant.
†Expressed as glucose, two hours after 400 mg glucose intraperitoneally
‡Determined on a liver extract prepared by grinding with sand in a mortar This procedure gives lower results than an extract prepared in a Waring blender, as in the androgen experiments, but the ratio of activity in the livers of control and tumour bearing rats is the same in both methods

It will be noted that in no instance was tumour growth inhibited, and histological examination of the tumours from the treated animals did not show any variation from the control group.

Discussion

The magnitude of a systemic effect is related to the size of the tumour (Part I). For this reason it is essential in any attempt to modify systemic effects that there be no difference in the growth-rate and size of the tumours in the control and treated groups.

Steroid hormones have been shown to affect the growth of mammary tumours in the human, and the response of the host to the tumour (Therapeutic Trials Committee, 1949). In the rat bearing the Walker 256 carcinoma there is no effect

on the tumour and only a slight influence on tumour-host relations. Haddow (1950) has directed attention to this discrepancy of the effect of chemotherapeutic agents in the laboratory and in the clinic.

The experimental demonstration that TCP is a more potent androgen than testosterone propionate does not mean that it would necessarily have a greater effect on hæmoglobin and liver catalase activity. Studies on the metabolic effects of the androgenic hormones do not permit yet of a correlation between action on enzyme systems and protein synthesis and the effects on the seminal vesicles and prostate of the castrated immature rat. Kochakian (1947) has demonstrated that some steroids with a very weak or absent androgenic activity may have a moderate influence on alkaline phosphatase and thymus involution.

The thymus involution in tumour-bearing rats implanted with pellets of testosterone propionate is the greatest that has been observed. The gland is reduced in most instances to a small thread-like structure. This marked involution is produced by the absorption of 1 mg. per day from the pellets, and exceeds that resulting from the injection of 2 mg. a day of the same steroid in oil.

Adrenal hypertrophy was reduced in degree by pellets of testosterone propionate, and it might be inferred that this was the result of inhibition of release of ACTH from the pituitary (Bartter, Forbes, Jefferies, Carroll and Albright, 1949; Faloon, Owens, Broughton and Gorham, 1950). If this were true, an increase in adrenal ascorbic acid might have been expected but was not demonstrated.

Unpublished experiments indicate that pellets of testosterone propionate do not alter the urinary excretion of nitrogen, sodium and chloride in tumour-bearing rats.

The extract of adrenal cortex was able to reduce the degree of anæmia but had no influence on the loss of liver catalase activity. It has been reported that an aqueous extract of the adrenal increases hæmoglobin in the rat (Yoffey and Baxter, 1946).

There appears to be some variation in the response of tumours to steroids of the adrenal cortex (Sugiura, Stock, Dobriner and Rhoads, 1950), but an inhibition of growth of the Walker 256 carcinoma has been reported (Ingle, Prestrud and Rice, 1950). This occurs in the presence of an inhibition of body growth more marked than in this series.

The failure to affect the adrenal by the LAC may be in the design of the experiment. The mean weight of the adrenal in the treated group is smaller and the cholesterol content higher than in the control. If the series were expanded to include larger numbers of animals the differences might become significant. Such a possibility is supported by the fact that LAC did have a sparing action on the loss of adrenal sudanophilia, which has been correlated with cholesterol (Sayers, Sayers, Fry, White and Long, 1944).

The failure to produce anticipated effects may be more fundamental, and may be due to an inadequate dosage of LAC. It has been estimated that the daily output of the adrenal of the rat is the equivalent of twenty-five millilitres of an aqueous extract of the adrenal cortex, based on the amount required to permit normal performance of the work test in the adrenalectomized rat (Ingle and Nezamis, 1948).

Summary

Tumour-bearing rats exhibit enlargement of the adrenal, with loss of ascorbic acid and cholesterol, atrophy of the thymus, diminution in liver catalase activity and progressive anæmia.

The thesis that rats bearing large tumours are in a state of hypofunction of the adrenal cortex requires further substantiation, and in any event such a state would not explain the observed systemic effects.

Pellets of testosterone propionate reduce adrenal hypertrophy in the tumour-bearing rat, and an extract of the adrenal cortex diminishes the degree of anæmia. No effect was noted on the growth of the Walker 256 carcinoma.

The author is indebted to Dr. H. P. Rusch of the McArdle Memorial Laboratory for donor rats carrying the Jensen sarcoma and the Walker

256 carcinoma, and to Mr. T. E. Dickinson, B.Sc., and Mr. D. G. Withers for technical assistance. The work was begun during the tenure of a British Council Scholarship at the Sir William Dunn School of Pathology, University of Oxford.

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DISCUSSION

BOYLAND: It is quite clear that the changes in liver catalase and hæmoglobin do not go together. Injection of suitable extracts of [illegible]

[illegible] shortage of liver catalase [illegible]

arginase in tumour-bearing animals.

BEGG: Greenstein supported that view.

HADDOW: I am always a little anxious that effects of the kind Dr. Begg described are not secondary. We may have minor degrees of infection which do not substantially hold up the tumour but maybe responsible for some of these effects. Have you any view on these possibilities?

BEGG: I think it's very difficult. We have tried to limit our results to cases where the tumours were the same size and of standard microscopic appearance. If you're studying tumour-host relations, you must keep to a standard size, otherwise you would get a greater or less amount of catalase simply from a larger or smaller tumour.

HERTZ: I think this matter of the mystery regarding the mechanism of death of cancer patients has been very much over-emphasized

eventually die, in how many would you expect to get hæmorrhage, intestinal obstruction and so on?

HERTZ: The experimental situation is different from the clinical.

BEGG: Clinically, although a large proportion of patients have had some metastases to the lung and some to the liver, there are many people who have much greater destruction from old tuberculosis and old cirrhosis and live quite happily.

PART IV

CLINICAL AND METABOLIC EFFECTS OF ACTH AND CORTISONE IN NEOPLASTIC DISEASES

STEROID HORMONES AND CANCER

KONRAD DOBRINER

THE relation of steroid hormones to the cancer problem has been investigated for more than 40 years in both animal and clinical studies. The results have been reviewed at intervals by a number of authors (Lacassagne, 1939; Allen, 1940; Gardner, 1947; Nathanson, 1947; Lipschutz, 1950). Even before knowledge of the structure and the physiological action of the steroid hormones was available, keen observations by Lathrop and Loeb as early as 1916 indicated that gonadal function was concerned in the ætiology of cancer. These workers reported that early gonadectomy caused a marked reduction in the incidence of spontaneous mammary cancer in mice. After the isolation of œstrone by Doisy and Butenandt in 1929, Lacassagne (1932, 1939) in 1932 found that administration of pure female sex hormone produced mammary cancer in male mice of high cancer strains. Since then, in a great number of investigations, the role of the sex hormones in the ætiology of cancer has been explored by the removal of the gonads as well as by the administration of male and female sex hormones. Gardner (1947) in his review of the steroid hormones in experimental carcinogenesis, summarized the present view by stating that œstrogenic hormones and genetic factors have independent ætiological significance in several types of experimentally induced lymphoid, mammary, testicular, and hypophyseal tumours of mice.

In the period from 1935 to 1942, the hormones of the adrenals have been isolated by Reichstein, Kendall, and *Wintersteiner*, who demonstrated that the adrenal hormones were closely related in structure to the male and female sex hormones (review by Reichstein and Shoppee, 1943). During the same period, it was established that the adrenal hormones were necessary for life maintenance, and in addition were intimately concerned with protein, carbohydrate and electrolyte metabolism (Ingle, 1950). They influence the lymphatic tissues in a very significant way. Dougherty and White (1943) demonstrated involution of lymphatic tissues when adrenal function was increased and showed that this effect was coincident with nitrogen loss (White, 1950). In the same period, Kenyon (1944) and Kochakian (1950) demonstrated that the sex hormones functioned in the anabolic phase of nitrogen metabolism, while Albright (1947) showed that the female sex hormones play an important role in calcium metabolism and bone formation. These were notable discoveries since they demonstrated that the biological activity of the "sex steroids" was more far reaching than their influence on the secondary sex characteristics. The metabolic function of these hormones emphasizes a dual role in their action.

In 1944, Heilman and Kendall demonstrated that an adrenal hormone, cortisone, produced regression of lymphoid tumour tissue in mice, and Murphy and Sturm (1943) found a decreased susceptibility to transplanted leukæmias under the influence of adrenal hormones. Dalton (1944) found evidence of morphological changes in the adrenal gland with the development of tumours. These observations indicated that steroid hormones influenced abnormal growth. It may be suggested that the animal does not produce enough hormone to prevent tumour formation and that this failure of hormone production is due to a disturbed or decreased adrenal function in these animals. This problem has not been well studied with laboratory animals, but in our own investigation, which I will discuss later at this meeting, we were able to show that adrenal function is abnormal in many patients with neoplastic

growth. In 1944, not enough adrenal steroids were available to test the concept that adrenal hormones would be useful in humans with disease of the lymphatic tissues. The hope of obtaining large enough amounts of the adrenal stimulating hormone, ACTH, the isolation of which was reported at this time by Li *et al.* (1943) and Sayers *et al.* (1943) failed because industry was engaged in the emergency of war and because there was no promise of a cancer cure.

There remained, however, the possibility that hormonal imbalance might be involved in the production and growth of tumours. This hypothesis was supported by the work of Woolley (1950), who observed that endocrine imbalance produced by gonadectomy resulted in a nodular hyperplasia of the adrenal cortex that in certain instances led to adrenal tumours. The nodular hyperplasia was accompanied by pituitary tumours in these animals. It is of great significance that the administration of either male or female sex hormones to these gonadectomized mice *before* adrenal hyperplasia occurred prevented the adrenal and pituitary tumours. This close relation of endocrine imbalance, tumour formation, *and prevention by steroid hormone administration*, was supported and extended by the observation of Gardner (1947) that in certain low tumour strains of mice, testicular, pituitary, lymphoid, cervical and mammary tumours occur after œstrogen administration and that the incidence of these tumours can be decreased by the simultaneous administration of testosterone. Consequently, the major conclusion may be drawn that carcinogenesis is dependent on hormonal imbalance of several origins, involving a disturbed function of the gonads, adrenals, and pituitary glands.

The evaluation of endocrine factors in the ætiology of human cancer is much more difficult, since experimental conditions cannot be made equal to those available with animals. The hormones are differently metabolized in humans and in lower animals, and it may well be that their function and interplay is similarly variable. That the steroid hormones play a role in the ætiology of human cancer is strongly

suggested by certain observations, for example, the failure of eunuchoids and eunuchs to acquire prostate cancer is probably due to the lack of testicular hormone production (Moore, 1944). The incidence of human cancer increases at the time of endocrine changes, especially near the menopause in females. The age incidence in human cancer may well be linked with demonstrable endocrine imbalance or deficiency.

We therefore investigated in patients with neoplastic disease whether there were quantitative and qualitative changes in steroid excretion, in order to establish the facts of hormone production (see literature cited on p. 222). The results were compared with those obtained from normal subjects of the same age group. We concluded from our results that a decreased gonadal function and an abnormal adrenal secretion existed when cancer was present. This abnormal glandular function was established in three patients more than three years *before* cancer was diagnosed. If we look at these facts, it is clear that the decrease of gonadal and adrenal hormones is of major importance in cancer and that to influence the disease, it is necessary to restore a more normal glandular function.

As a consequence, substitution therapy was applied in the hope of restoring a normal hormone balance, and thus influencing abnormal growth. The administration of œstrogen and androgen in cancer of the breast (Nathanson, 1947) and the withdrawal of hormones by gonadectomy and adrenalectomy in cancer of the prostate (Huggins, 1946, 1950) have been reported as beneficial in these diseases. The mechanism of action on the abnormal process may be partly direct local action on the tumour as well as the profound general effect of these hormones on proteins and electrolyte metabolism in normal and abnormal tissues.

Another factor to be considered is the change these hormones cause in the endogenous production of the glands, which may in turn influence the neoplastic growth. Changes in endogenous production of hormones can be illustrated by three examples (Dobriner and Lieberman, 1950): (1) The prolonged

administration of testosterone changes the endogenous production of the precursors of androsterone and ætiocholanolone, since the urinary level of these metabolites decreased markedly after the treatment (Fig. 1). (2) The prolonged administra-

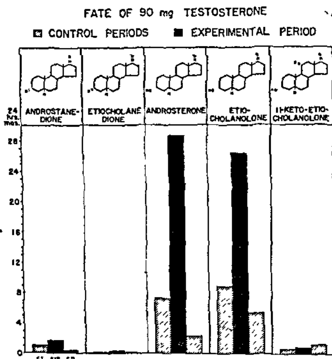


FIG. 1. The quantitative excretion of five steroid metabolites

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=post-testosterone control period.

tion of very small amounts of adrenal extract decreased the production of both 11-desoxy and 11-oxygenated hormones (Fig. 2). (3) Removal of the testes decreased the excretion of *androsterone* and *ætiocholanolone*, the metabolites of the

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apparent from the known reactions of these hormones on lymphoid tissues that the major attempts in humans should be directed toward disease of the lymphatic organs. The clinical aspects of this work were conducted by Dr. Pearson, Eliel, Burchenal and others* (Pearson *et al.*, 1949; Pearson and Eliel, 1950, 1951; Pillers *et al.*, 1951). The complete metabolic balance studies were made by Dr. Pearson, Eliel and others in the department of clinical investigation

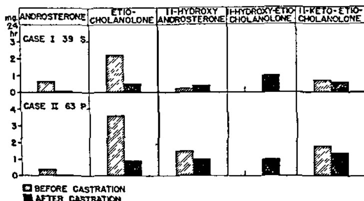


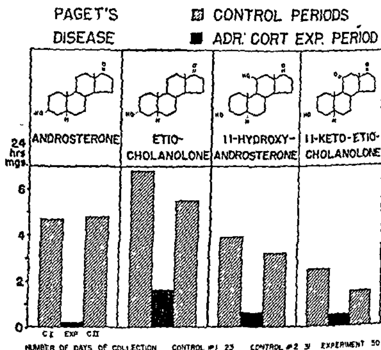
FIG. 3. The effect of orchiectomy on the excretion of five steroid metabolites by two men with cancer of the breast The values are expressed in milligrams per 24 hours.

(Pearson and Eliel, 1951), and in many of these patients the steroid excretion patterns were established, as I shall show later in this conference.

About 70 patients have been treated with 100 to 500 mg. of cortisone, or 100 to 400 mg. of ACTH per day for various lengths of time. Marked temporary regressions occurred in chronic lymphatic leukæmia, lymphosarcoma, acute lymphatic or granulocytic leukæmia, plasma cell myeloma and Hodgkin's disease. No effects were observed in patients with chronic myelogenous leukæmia, acute microcytic leukæmia, Ewing tumour, neuroblastoma, malignant melanoma and

*Dr. Burchenal reports the clinical findings on p. 198

male sex hormone as well as adrenal steroids (Fig. 3). These compensatory changes in hormone production resulting from the administration of steroids or from the withdrawal of one of the producing glands are probably mediated through



[illegible] of five steroid metabolites

changes in production of trophic hormones in the pituitary gland.

As the adrenal hormones as well as ACTH have been available since the fall of 1949, the opportunity was at hand to investigate their effect on neoplastic growth. It was

Tannenbaum (1947) in particular has emphasized the role of inanition in the growth rate of tumours. Tumour growth under high caloric intake can be viewed as a specific hormone starvation of neoplastic tissue, in that a large amount of the hormone participates in the elevated metabolism of tissues and is thus not available to the neoplasm. This is certainly suggested by the experiments of Pearson and Eliel (1950). If the hormonal supply were sufficient for the whole animal or could be applied *only* to the tumour tissue, it is easily possible to imagine the tumour shrinking to the vanishing point, even with excessive nitrogen intake. Conversely, if there had been enough hormones available at all times, the tumour might never have had the opportunity to come into existence.

From the clinical results and from the knowledge of hormone action, we can draw three significant conclusions: (1) Adrenal hormones, through their effects upon nitrogen metabolism, can be used to interfere with the progress of a tumour. If endogenous steroid production is adequate, or proper substitution therapy is undertaken, it is quite possible that certain tumours would not develop. (2) The change in hormone production and balance at present can only be detected in its subtle initial stages by study and examination of the urinary steroids. These substances therefore provide one of our few indices of physiological change associated with tumour incidence. (3) The "sex" hormones may be implicated in the cause of some tumours, especially neoplasia associated with reproductive organs. These same hormones unquestionably influence the course of the tumour's progression, and further study of their action in disease offers hope for a better therapy and possibly the prevention of abnormal growth.

I would like to express my deep appreciation to Dr Thomas F. Gallagher for his valuable suggestions and generous assistance in the preparation of this manuscript.

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several types of sarcoma and carcinoma. No differences have been observed in the clinical effects of administered cortisone or the hormones produced by the patient's own adrenals under the stimulation of ACTH, *provided that the adrenals responded adequately to the stimulus.* The therapeutic effects, although temporary, were thus restricted to the lymphatic tissues, and this was not an unexpected result in view of the biological action of the adrenal hormones. The necessity for large amounts of hormones was also not surprising, since it seems that the *normal* adrenal produces much larger amounts of hormones than were anticipated from purely a *priori* consideration.

There are three points in the metabolic studies of Pearson and Eliel that deserve special comment. *First:* They found that adrenal hormone administration in chronic lymphatic leukæmia with large tumour masses caused a disproportionate excretion of phosphorus in comparison with nitrogen. This finding is of great importance. It proved objectively that lymphatic tumour tissue was being destroyed, since lymphoid tissues are characterized by a high ratio of phosphorus to nitrogen. *Second:* After the combined administration of cortisone and testosterone in several patients with chronic lymphatic leukæmia with large tumour masses, the tumours shrunk considerably, and the patients remained in nitrogen balance. The phosphorus excretion continued at a high level, however, and the patients were in negative phosphorus balance. This observation indicates that the adrenal hormone under certain conditions *acts specifically* on tumour tissue, since the tumour regressed whereas the catabolism of other tissues was counteracted by the anabolic action of testosterone. *Third:* It would seem that the beneficial effect of cortisone is dependent on dietary intake. One patient, who showed a decrease of tumour masses during the administration of cortisone and exhibited a markedly negative nitrogen and phosphorus balance, was given twice the amount of food. The nitrogen and phosphorus balance then became slightly positive, and no further shrinkage of the tumour occurred.

DOBRINER: There was a slight increase in phosphorus excretion but it was not significant. It is a difficult thing to understand because tissue is being broken down and yet the products are not excreted. They must be used for rebuilding.

BOYLAND: It would be interesting to know the nature of the excess nitrogen excretion. One might expect it to be derived from nucleic acids, and that there would be an excess of uric acid.

DOBRINER: There is in some patients and not in others.

KELLIE: It seems to me that the importance that was formerly laid [illegible]

DOBRINER This is somewhat outside of my personal field

BEGG: Nucleoproteins are synthesized but uric acid comes from a breakdown of the nucleoprotein residues.

[illegible]

BEGG: Yes, but via the purine system We can undoubtedly say that [illegible]

lished.

DOBRINER· It will take a long time because this is very tedious and time-consuming work.

KELLIE: Is there any definite information whether Compound F has effects which are not present with Compound E, or vice versa? Or can one assume that Compound E will be reduced to Compound F?

DOBRINER· I will show this to you later. It is a very simple metabolic change and it seems to occur all the time. The substance produced by the adrenals under ACTH stimulation is certainly not Compound E; it is Compound F

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DISCUSSION

[illegible]

excretion there:

with a million cells intraperitoneally the control mice develop tremendous spleens and large lymph nodes. The spleen from one of these untreated animals weighed 1,550 mg. The spleen from a mouse which had been treated for 17 days with cortisone at 50 mg. per kg. three times daily (which is a toxic dose) weighed only about 65 mg. The nodes could not be found, and sections of the spleen and liver showed no leukæmic infiltration. This strain of leukæmia in the C_{58} stock of mice (which is not our standard strain) has been of use, and we hope to be able to get a dose which will prolong the survival time of these animals.

On the other hand, acute leukæmia in man is a different story. Dr. Dobriner has given you an account of the reasons that led up to the trial of ACTH in acute leukæmia by Dr. Pearson and, I believe independently at about the same time, by Dr. Farber of Boston. Today I am speaking here, partially for myself, but mainly for Dr. Pearson. Dr. Pearson has treated some 31 cases of acute leukæmia and did the original work and the metabolic work on the study of this disease. Our group has been interested in it more from the therapeutic trials aspect, and we have treated an additional 21 cases with cortisone, so that altogether we have treated some 51 cases of acute leukæmia. In Dr. Pearson's group 20 children were treated with these two compounds, and nine of those had good remissions.

Of 10 adults, four responded well. In our series of 15 children, nine did well, and of six adults, only one responded well. In other words, out of 35 cases in children, there were 18 good responses; out of 16 adults, five good responses.

I will discuss mainly our own results now. We got real remissions, hæmatological remissions with the peripheral blood returning approximately to normal, liver and spleen going down to normal size, nodes going down, and with a marrow which returned approximately to normal. I do not say that at the best of the remissions one couldn't find a leukæmic cell. I'm sure that, if a hæmatologist looked at it and knew it was a case of leukæmia, he could find leukæmic cells in that

ACTH AND CORTISONE IN ACUTE LEUKÆMIA IN CHILDREN

J. H. BURCHENAL

I AM going to talk mainly about the therapeutic effects of cortisone and ACTH on acute leukæmia.

I would like to say a word about the advantages of using acute leukæmia for an assay method, a method of testing compounds. Dr. Huggins mentioned this morning that the clinician always has difficulty in assessing the value of a compound in the patient unless it is a truly curative compound, which obviously none of these are. Biopsies can be done occasionally, but regular biopsies are hard to get unless you have a very co-operative patient. In acute leukæmia, by sticking the patient's finger or by perhaps doing a sternal puncture, you can get a biopsy of the tumour at any time that you wish. Also you have a disease which runs a rather acute, fulminating course, which has some spontaneous remissions, but not very many, and which rarely shows two spontaneous remissions in an untreated patient. Moreover, it occurs fairly commonly in children, and children appear to be able to stand the effects of substances such as ACTH and cortisone better than older people, particularly the aged.

There have been difficulties in using mouse leukæmia as a screening procedure for the steroids because the leukæmia which we have been using has been very acute, and has not been affected by ordinary doses of cortisone; with the maximum tolerated dose of cortisone or ACTH we cannot prolong the life of these animals. However, these compounds do have some effect on the leukæmia, because if we give large doses, supralethal doses, the animals will die at the same time as the control animals, but they will not die of leukæmia; they will have no infiltration. Nineteen days after inoculation

Fig. 1. *Marrow of patient with acute leukæmia, after treatment with 50 mg. cortisone per day for 28 days*

Fig. 2. Same patient as in Fig. 1. After treatment with 400 mg. cortisone per day

marrow, but they have decreased to a very small percentage. On the other hand, I don't believe that a hæmatologist seeing those marrows, without knowing that the patient had leukæmia, could make the diagnosis. In a few cases, however, we have had hæmatological remissions in which the marrow improved markedly, but the patient did not. I've not seen that with other drugs in acute leukæmia.

Figs. 1 and 2 show an example of what we mean by a good hæmatological remission. Fig. 1 shows the marrow of a patient with acute leukæmia after he had been treated with a little cortisone. His marrow was approximately that way before any treatment at all. He had 50 mg. a day for 28 days, an insufficient amount so far as he was concerned. As you can see, his marrow was made up all of one type of cell, stem cells of acute leukæmia.

After he had been treated with 400 mg. of cortisone a day the cell picture (Fig. 2), instead of being uniform as it was before, showed nucleated red cells, myelocytes, metamyelocytes, and polymorphonuclears, the more mature cells of the myeloid series.

I would like to discuss a case that is typical of those we have been treating. This is of a seven-year old girl who had been treated with other drugs for some time before and had responded to them, but had eventually failed to respond to anything. Her bone marrow showed 87 per cent stem cells. Her white count was rather low, down around 5,000, but the percentage of abnormal cells was almost 100 per cent. She was then treated with cortisone. There was a slight rise at first in the white count and then a fall, and the thing that is really important is that the percentage of stem cells decreased to 10 per cent, and that the marrow showed essentially a normal picture. Stem cells, of course, should not be present in a normal marrow in any appreciable quantity, but it's very difficult to differentiate between lymphoid cells and stem cells, particularly in children, so that it is possible that that figure may be 10 per cent lymphocytes, which is not too far from normal. During the time that she was improving the

eosinophils fell, and the reticulocytes rose slightly to about 5 per cent. She had one transfusion which built up her hæmoglobin and after that she maintained it quite well. However, after stopping treatment for a short while, there was a rapid reversion to an abnormal marrow picture. She was started on treatment with antifolics, to which she was resistant and had never responded, and there was no more response. The marrow became worse. She was brought in, transfused, because at that stage she was in rather poor shape, and then she was treated again with cortisone. The marrow again responded, going from 90 per cent abnormal cells down to 11 per cent, with a fall in the eosinophils and with a marked rise in reticulocytes, up to about 15 per cent. After her second course, she was in quite good shape, and had not shown too many undesirable side effects. She put on some weight, but she did not show particularly spindly legs, nor a very large abdomen, and her face, although fuller, was not as full as it might have been.

We have seen remissions lasting anywhere from one to twelve weeks. In many of these cases the marrow relapses although the patient still feels well, perhaps for as long as a month after. We usually start treatment, if possible, as soon as a real relapse in the marrow shows up.

We have usually given ACTH four times daily in doses of 50–100 mg. a day for children and 100–200 mg. a day for adults, but we have gone up to 400 mg. a day in some patients, and above that in one or two. ACTH is given four times daily because it has been shown that ACTH goes in and out of the adrenals fairly rapidly.

In Dr. Pearson's group cortisone has been given the same way, every six hours. The dosage has been a little higher, running from 50 mg. to 100 or 200 mg. in children, and in adults, from 200 to 400 mg. in a total daily dose. Because we were interested in treating some patients as outpatients because of lack of beds, we tried giving these doses in a single daily injection and I think that our results have been reasonably good. We have not titrated it down very carefully to

occurred particularly in children. It comes on sometimes rapidly, sometimes after a considerable dosage, and we have found no way of getting away from it. It seems that if the blood pressure goes up to high levels or if episodes of hypertensive encephalopathy with loss of consciousness intervene, it is best to stop the drug.

Psychoses have been seen in a certain number of patients and we feel that if there is any real mental aberration, it is best to stop the drug. Some of these patients have committed suicide. Others have died during treatment, but presumably of their disease rather than of the psychosis. Others are still being treated for the psychoses with no sign of improvement. We have a psychiatrist who watches these patients fairly closely, and when he feels there is any abnormality appearing, we generally stop treatment. Sometimes, however, the psychoses come on very rapidly. Patients who are watched very closely may seem a little agitated one morning and suddenly commit suicide the next day. We have not been impressed by psychiatric changes in children as yet. It's a little hard to assess that because the children come from all sorts of environments. Their personality changes a certain amount while they're in a ward, even without treatment, because they are away from their normal environment and they get bouts of homesickness. We have not yet seen any frank psychoses in children.

We have been very impressed by the occasional incidence of overwhelming infections in these patients, mostly patients who have had prolonged treatment with one of the steroids. Of course, in leukæmics one normally expects to get infections, but not in a leukæmic whose bone marrow has gone back to normal, as in some of these patients who had good hæmatological remissions, but no clinical remissions. Dr. Pearson's and our groups both feel that there is very definitely, in some of these cases, an increased susceptibility to infection. If there is an infection and if the antibiotics which are used in large doses don't seem to be able to handle it rapidly, then we feel it is best to stop the steroids.

make sure that a patient who responds well to 200 mg. in a single dose would not respond to perhaps 100 mg. if the dose were divided, and I think it's very possible that divided dosage may be better. I can say, however, that in a child given a single daily dose of 150 or 200 mg., definite beneficial effects can be obtained. In some of the patients who have died under treatment, we have seen large depots of cortisone in the muscles. Since cortisone is relatively insoluble, probably one gets a depot effect, and a single injection a day may be sufficient.

Dr. Dobriner has gone over the undesirable side effects that occur with these drugs. Sodium retention with water retention and œdema is certainly important. From a therapeutic point of view that can be handled quite well by putting the patient on an almost salt-free diet, or at least a low sodium diet.

Weight gain, aside from œdema, occurs if these patients are allowed to eat as much as they want. We try to calculate what the child should eat if he were in normal health, and put him on a diet limited to that number of calories. Before he's getting cortisone or ACTH he won't eat that much, but after he gets these drugs he rapidly develops a ravenous appetite and reaches the ceiling imposed by his diet. Then if you can watch the other children and make sure he doesn't steal food from them, on that limited food intake and low sodium diet there is usually no weight gain.

Metabolic alkalosis has to be watched for, but when it develops it can be corrected by the administration of potassium chloride.

In patients who have been treated for a long period of time with ACTH or cortisone we have seen development of Cushing's facies, a certain amount of hirsutism, and acne. These symptoms, although bothersome to the patient and occasionally to the family, have not been a contra-indication to further therapy.

There are, however, three difficulties that have not been surmounted as yet. The first is a rise in blood pressure, with or without hypertensive encephalopathy. That has

for about four weeks and then he came in and the marrow was all bad again. He was started on the same dose of ACTH that he had before, and there wasn't any change in the marrow at all. He was then given larger doses of ACTH, up to 400 mg. per day and I think he was also given cortisone just before death, but there was no improvement the second time, no change in the bone marrow, no improvement in nucleated red cells. The differential, which went bad a little more slowly than the marrow differential, still stayed completely bad, and the white count, which had started going up in his last relapse, continued to go right on up and was around 870,000 at the time of death. So that there was absolutely no clinical response, although metabolically he was showing some response, as shown by his increased excretion of steroids.

Now we come to the practical value of these compounds. We are looking for a compound that will work when the folic acid antagonists do not work, because we have a large number of patients under treatment constantly, and we know that sooner or later the folic acid antagonists are going to cease to work. An example of this is L.D., a five-year old boy who had been treated since December 1948 with folic acid antagonist and had repeated responses, but finally reached a stage in March 1950 where he was totally resistant to the antifolics. He was then treated with 150 mg. of cortisone a day. He had an episode of hypertensive encephalopathy which alarmed us; at first we thought he had had a cerebral accident, but he had not, and we stopped treatment for a short while and then started again. His blood pressure did not go up too markedly, and we felt that it was do or die with him so we carried on the therapy. Eventually he got a response. His stem cells decreased down to about 30 per cent. That is not a perfect marrow by any means, but the percentage of erythroid elements increased to 55 per cent of all the marrow cells, and he built hæmoglobin. He had a small transfusion, but the rise in hæmoglobin was not due to that; it was due to his building new red cells of his own. His reticulocytes

As to the repeatability of these remissions in children, we have had six who have had a second course of therapy and who have responded. Two of these, from one of whom I showed you a marrow slide, had just as good remissions with the same amount of the drug as they did the first time. On the other hand, four others required considerably more cortisone or ACTH the second time. We have pushed some of them into partial third remissions, but generally there is a falling off in the relative completeness of the remission and the ease with which it can be attained. They seem to develop a refractory state to this drug faster than a similar type of patient would do to the folic acid antagonists.

In adults, on the other hand, we have had two patients with excellent results the first time, who were treated a second time and neither showed any beneficial effects. I would like to discuss one of them, case RR, in more detail. He showed a beautiful response the first time; the second time, although he had had a metabolic response, his leukæmia was not affected. On his first course of therapy he started out with a white count of 100,000 cells, and after four or five days' treatment with ACTH his count was down to 2,000. This is one of the interesting things about ACTH. Ordinarily in treating chronic leukæmia with the nitrogen mustards or acute leukæmia with the antifolics, if we get a drop from 100,000 down to 2,000, we would be alarmed and would stop therapy, but this therapy was carried right on and the count levelled off at 2,000, didn't go down to 200 or 20 cells, and the patient got a tremendous and very rapid clinical betterment. He was a young doctor who knew the situation and perhaps there was some psychical aspect, though it was hard to see any. He had considerable bone pain when he came in. Within 12 hours of getting his first injection of ACTH that had eased. There was rapid improvement in the myeloid cells of the peripheral blood and in the marrow. The erythroid activity increased from practically nothing up to 60 nucleated red cells per 100 white cells.

After the drug was stopped the marrow remained normal

some transient decrease in the nodes or in the masses, which is very real but which is not as prolonged as with X-ray therapy. In the cases where the patient is X-ray resistant and moribund, then this agent is no better than HN2.

We also tried Compound A, 125 mg. given four times per day. It was administered for 22 days in a case of acute leukæmia. The patient showed an increased weight and œdema, but there were no beneficial effects.

We are now trying Compound L. It has been administered in dosages of 150 mg., twice daily, but we have no results as yet.

DISCUSSION

GARDNER: The laboratory tests have been on transplanted leukæmia

very large numbers of mice.

GARDNER: *Do you think that there might be a certain point of similarity between the resistance to the second and third treatment in the human cases and the prolonged survival of the cells in the animals that had transplanted leukæmia?*

BURCHENAL: I don't know. But we do have experiments underway with the Patterson lymphosarcoma and with the Wagner osteogenic

BEGG. In what per cent of cases did you give transfusions and antibiotics?

BURCHENAL. We tried to stay away from transfusions as much as possible, although on the other hand, if the patient looked in bad shape, we didn't hesitate to give them. We've got a base line of 150 cases that were treated with transfusions and a certain number of antibiotics, but there always is a possibility that if you put a large amount of blood in there that you may get some temporary remission. We have had to use antibiotics fairly frequently because of infection.

BEGG: You have some cases there in which you have response without transfusion?

went up markedly. When I left we had just started him off on antifolic therapy, hoping that he might respond again to it.

We have also tried the converse, using the antifolics on patients who have become resistant to cortisone or steroid therapy. Three of those five or six patients have responded well to the antifolics.

I will try to compare briefly the values of these compounds with some of the known therapeutic agents in cancer. X-ray, of course, is useless in acute leukæmia, and HN2 and the various mustards are of no real benefit. The antifolics are useful in some cases; in children 30–40 per cent remissions can be achieved. With the antifolics there is very little beneficial effect in adults, and it takes longer to get results. It would appear that the steroids and ACTH work faster in the acute leukæmias, and I believe that you probably have more chance of helping a very sick patient with ACTH or cortisone than you have with the antifolics. Once you get him out of his sick situation, and if you do get a remission with either compound, I think you have more chance of getting many remissions with the antifolics than you do with the steroids. With the latter the remissions are not likely to persist as long or to be as repeatable.

There is a marked contrast between chronic lymphocytic leukæmia and the acute leukæmias As Dr. Dobriner has told you, if you treat a case of chronic lymphocytic leukæmia with *cortisone or ACTH, the spleen decreases in size, the* nodes decrease, the liver decreases, but the white count goes up tremendously, from 100,000 or so to as high as 800,000. We've seen one case who went from 700,000 to 1,200,000. On stopping the dosage, surprisingly enough, the count falls. On the other hand, this drug does seem to have some beneficial effect in patients who, if treated with X-ray, might well develop *thrombocytopenia, anæmia, and so on.*

There seems to be some suggestion of stimulation of the erythroid elements, so it has usefulness there compared with HN2. In Hodgkin's disease and in lymphosarcoma there is

some transient decrease in the nodes or in the masses, which is very real but which is not as prolonged as with X-ray therapy. In the cases where the patient is X-ray resistant and moribund, then this agent is no better than HN2.

We also tried Compound A, 125 mg. given four times per day. It was administered for 22 days in a case of acute leukæmia. The patient showed an increased weight and œdema, but there were no beneficial effects.

We are now trying Compound L. It has been administered in dosages of 150 mg., twice daily, but we have no results as yet.

DISCUSSION

GARDNER. The laboratory tests have been on transplanted leukæmia rather than on the spontaneous disease in all cases so far, have they not?

[illegible]

very large numbers of mice.

GARDNER: *Do you think that there might be a certain point of similarity between the resistance to the second and third treatment in the human cases and the prolonged survival of the cells in the animals that had transplanted leukæmia?*

BURCHENAL: I don't know. But we do have experiments underway with the Patterson lymphosarcoma and with the Wagner osteogenic

[illegible]

BEGG: In what per cent of cases did you give transfusions and antibiotics?

BURCHENAL: We tried to stay away from transfusions as much as possible, although on the other hand, if the patient looked in bad shape, we didn't hesitate to give them. We've got a base line of 150 cases that were treated with transfusions and a certain number of antibiotics, but there always is a possibility that if you put a large

[illegible]

BURCHENAL: Some cases in our series have had no transfusion and [illegible]

[illegible]

that it was neoplastic tissue, or at least lymphoid tissue, that was being destroyed. Wouldn't you think that was so, Dr. Dobriner?

DOBRINER: Yes. I think it is good metabolic evidence.

BURCHENAL: In chronic lymphocytic leukæmia we find that we can continue to get remissions on repeated treatment. After Dr. Pearson treats his chronic lymphocytic leukæmias and gets a response, they do well for about three months and then the nodes start coming up again He has treated them again and again. One of them has been treated for 15 months at least, and the responses are approximately as good now as they were to start out with. These were all adults. It seems rather surprising to me that the chronic lymphocytic leukæmias should continue to respond just as well, whereas the acute leukæmias in adults do [illegible]

However, we are trying now to wait a little longer instead of giving [illegible]

[illegible]

STOCK: No.

[illegible] the cells of the myeloid and [illegible]

in these cells.

[illegible]

definitely stimulated, as shown by the reticulocytes.

BEGG: What about the effect of antibodies to ACTH? Is there [illegible]

[illegible]

effect. It looks as though the resistance there were right in the leukæmic cell. There have been reports in the literature of animals getting resistant to it, but as far as I know this has not been demonstrated in man.

BOYLAND: Have you tried simultaneous treatment with antifolic compounds and cortisone?

BURCHENAL: We have not tried that to the full, because we felt that if we did get a response we wouldn't know which it was due to. We tried it out and it hasn't seemed to be any better than either one. Dr. Farber does treat a lot of his cases that way. He treats them with various regimes. One time he treats for perhaps 5 or 6 days with ACTH, and then puts them over on the antifolic and another time he will treat with the two of them simultaneously.

SOMMERVILLE: I was surprised to hear that you got hypertension with both cortisone and ACTH. In cases where you gave ACTH for prolonged periods, did you find that the electrolyte change that was remarked earlier in potassium output and chloride retention was maintained?

BURCHENAL: We usually put most of the patients on potassium chloride.

SOMMERVILLE: Does ACTH cause hypertension in normal people and cortisone not?

DOBRINER: No.

BOMFORD: What effect do these substances have on the bone marrow of patients without this particular condition?

BURCHENAL: I can't tell you. They have used it in pernicious anæmia without leukæmia, and I believe there is some improvement in the erythropoietic elements in cases of Hodgkin's disease, where there is no real damage to the bone marrow.

BEGG: Hench and his group reported in the *Archives of Medicine* in February, that they definitely got improvement in hæmoglobin in their rheumatoid cases.

BOMFORD: There was no depression of white cell formation?

BURCHENAL: I don't think so. You very seldom get an aplastic marrow as you do with the mustards or with X-rays.

LYONS: Did you get any inflammatory reactions at the sites of the injections, especially after the second or third injection of ACTH?

BURCHENAL: It depends on the patient. We have seen patients who could tolerate large doses of it for a long period of time without any trouble at all, mainly children with very little musculature.

LYONS: Do you try sensitivity reactions before you start the patient?

BURCHENAL: No, we haven't done that.

LYONS: I wondered if it could be connected with the development of antibodies, as Dr Begg suggested.

BURCHENAL: I think it is more likely to be a question of poor absorption in some of these patients.

LYONS: Do you recover any of this ACTH in the urine?

DOBRINER: We have never tried

ADRENAL FUNCTION AND STEROID EXCRETION IN NEOPLASTIC DISEASE

KONRAD DOBRINER

This morning I have discussed the correlation of steroid hormones with cancer. I pointed out that the study of steroid hormone production in normal and diseased states is possible by a detailed investigation of steroid excretion in urine, if one makes the reasonable assumption that steroid excretion is a measure of the hormone production of the adrenals and gonads. Since 1940 our investigations have been directed toward the development of methods for the study of steroid excretion patterns, with the aim of understanding the relationship between endocrine function and diseased states, with special emphasis on neoplastic disease (Dobriner, 1948, Reifenstein, Homburger, and Dobriner, 1950; Dobriner and Lieberman, 1950). We have thus far developed our procedure to its present use which requires a one day urine sample. By means of careful chromatography after chemical separation of the principal classes of steroids, we are able to obtain all or very nearly all the ketosteroids as single pure compounds or binary mixtures (Dobriner, Lieberman and Rhoads, 1948; Lieberman *et al.*, 1948; Lieberman, Fukushima and Dobriner, 1950). By the systematic application of infrared spectrometry on a micro scale, we can define the pure compounds or simple mixtures (Dobriner, Lieberman, Rhoads, Jones and Williams, 1948; Jones and Dobriner, 1949). Quantitative analysis by microcolorimetry then permits the determination of the amount of each steroid excreted during the twenty-four interval.

If we examine the steroid excretion patterns of normal males and females (Fig. 1) we find that there are four compounds that make up the major part of the ketosteroids.

Androsterone and its isomer ætiocholanolone are two metabolites of gonadal and adrenal hormones of the C-11 deoxy type like testosterone and compound S (Reichstein), whereas 11-hydroxyandrosterone and 11-ketoætiocholanolone are derived from adrenal cortical hormones with a C-11 oxygen

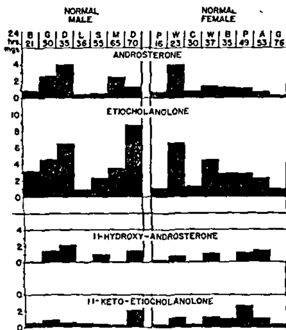


FIG. 1. Steroid excretion patterns of four compounds in normal male and female subjects.

function, like compound F and cortisone. In addition to these major metabolites, small amounts of many other steroids are excreted, including compounds F and E. In normal subjects there are no qualitative deviations from the normal pattern, but the amount of each compound varies somewhat in different individuals, just as there are *quantitative* differences in any metabolic process. One may say that the

patterns indicate the *individual* hormone production and that this varies between individuals just as there are differences in height, weight, or temperament.

The steroid pattern is quite abnormal in patients with neoplastic disease, as seen in Fig. 2, where a comparison has been made with the excretion levels of normal subjects in

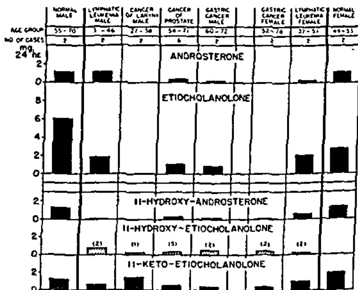


FIG. 2. Comparison of steroid excretion patterns in patients with neoplastic disease, and in normal subjects of the same age group

similar age groups. In contrast to the pattern shown for the normals (Fig. 1), there are both *quantitative* and *qualitative* differences in steroid excretion, whereas in the normals *only quantitative* changes are found. The decrease in the steroid excretion of cancer patients is especially marked with 11-deoxy type of steroids; for example, androsterone and ætiocholanolone were not excreted by male patients with cancer of the larynx nor by female patients with gastric cancer. In the other types of neoplastic growth, the excretion of these two

compounds was significantly decreased. A similar but somewhat smaller decrease in the excretion of 11-oxygenated compounds (11-hydroxyandrosterone and 11-ketoætiocholanolone) was observed. The *qualitative difference* is of much greater significance, since in the patients with neoplastic disease a *new compound is excreted*, 11-hydroxy-

COMPOUND F	COMPOUND S	TESTOSTERONE	
			HORMONES
11-OH-ETIOCHOLANOLONE	17 OH-PREGNANOLONE	ETIOCHOLANOLONE	METABOLITES
11-OH-ANDROSTERONE		ANDROSTERONE	

Fig. 3 Metabolic relations of hormones and their urinary metabolites.

ætiocholanolone, which from its chemical structure appears to be a metabolite of adrenal cortical hormones with a C-11 oxygen function. The metabolic relations of precursor hormone and excreted steroids are shown in Fig 3. 11-Hydroxyætiocholanolone was found in only two instances in a survey of 28 normal males and females. The two "normal" persons are over 70 years of age and have as yet no detectable evidence of neoplastic or other disease. Since the compound

occurs in the urine of some patients with essential hypertension and rheumatoid arthritis, its presence is not specific for cancer. 11-Hydroxyætiocholanolone is consistently excreted by patients with Cushing's syndrome, a disorder due to adrenal cortical hyperfunction with increased production of adrenal hormones of compound F and E type. The compound was also observed in the urine after stimulation of the adrenals by ACTH and after administration of large amounts of cortisone. However, 11-hydroxyætiocholanolone was not found in the urine of patients with the adreno-genital syndrome, another disorder characterized by the production of large amounts of adrenal hormones. The metabolic and clinical significance of the excretion of 11-hydroxyætiocholanolone is that in patients with neoplastic disease there is an abnormal adrenal function, somewhat reminiscent of the Cushing syndrome in the type of steroid produced. Whether this is only a derangement in hormone production, or whether there is in addition a faulty metabolism of a normal hormone, must be decided by further investigations with labelled hormones. We believe, however, that our results definitely indicate a very pertinent role of the adrenal gland in cancer, as I discussed in some detail this morning. In addition to the abnormal adrenal function, there is a very marked decrease in gonadal hormone production, to such an extent that in certain instances no trace of the metabolites of hormones like testosterone can be found in urine. This decrease or lack of male sex hormone production suggests that in certain cancer patients we are dealing with a condition very similar to physiological castration.

Two problems then arise: are these changes in adrenal and gonadal function connected with the cause of the disease, or is the abnormal hormone production and metabolism a conseque[illegible] of the disease? Both questions are difficult to
lo[illegible] ht that debilitation, so
laryn[illegible] have an influence on
other typ[illegible] is *not* the principal
[illegible] is well illustrated

in Fig. 4, where excretion patterns of patients with prostatic cancer are shown. This group of patients with cancer of the prostate was made up of men with and without debilitation. In all patients, independent of debilitation, the two major

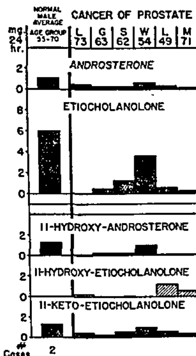


FIG. 4. Steroid excretion patterns in patients with cancer of the prostate and normal subjects of the same age group.

urinary metabolites androsterone and ætiocholanolone were markedly decreased in amount. The abnormal metabolite, 11-hydroxy-ætiocholanolone was present in all except one of these patients. I could give you many other illustrations to support the statement that general debilitation is not the cause of these changes in steroid excretion in cancer patients.

occurs in the urine of some patients with essential hypertension and rheumatoid arthritis, its presence is not specific for cancer. 11-Hydroxyætiocholanolone is consistently excreted by patients with Cushing's syndrome, a disorder due to adrenal cortical hyperfunction with increased production of adrenal hormones of compound F and E type. The compound was also observed in the urine after stimulation of the adrenals by ACTH and after administration of large amounts of cortisone. However, 11-hydroxyætiocholanolone was *not* found in the urine of patients with the adreno-genital syndrome, another disorder characterized by the production of large amounts of adrenal hormones. The metabolic and clinical significance of the excretion of 11-hydroxyætiocholanolone is that in patients with neoplastic disease there is an abnormal adrenal function, somewhat reminiscent of the Cushing syndrome in the type of steroid produced. Whether this is only a derangement in hormone production, or whether there is in addition a faulty metabolism of a normal hormone, must be decided by further investigations with labelled hormones. We believe, however, that our results definitely indicate a very pertinent role of the adrenal gland in cancer, as I discussed in some detail this morning. In addition to the abnormal adrenal function, there is a very marked decrease in gonadal hormone production, to such an extent that in certain instances no trace of the metabolites of hormones like testosterone can be found in urine. This decrease or lack of male sex hormone production suggests that in certain cancer patients we are dealing with a condition very similar to physiological castration.

Two problems then arise: are these changes in adrenal and gonadal function connected with the cause of the disease, or is the abnormal hormone production and metabolism a consequence of the disease? Both questions are difficult to answer. There seems to be no doubt that debilitation, so often a consequence of cancer, can have an influence on hormone production. That debilitation is *not* the principal reason for the change in steroid excretion is well illustrated

in her urine (period I). We have followed the steroid excretion pattern now for more than six years (period XI) after the recognition of her tumour, and in each period the abnormal steroid was present. It is now eight years after her mastectomy and no recurrence has been observed. This patient has not lost weight since the first urine collection, and, with the exception of increasing age, enjoys perfect health. This is a very good instance where debilitation can be excluded as the reason for an altered steroid excretion. I would like to mention another patient with aplastic anæmia of many years duration. She excreted the abnormal steroid 11-hydroxyætiocholanolone in a collection period four years before her death at a time when no cancer was diagnosed. The autopsy revealed, however, the existence of a cancer of the lung. These two instances certainly prove that the adrenal abnormality was present when cancer was not diagnosed. This adrenal disturbance seems to be present where early cancer may not be detected clinically, and may possibly exist in cases where cancer will develop at a later date. If this latter point could be proven, then *the cause of cancer would involve* a steroid hormonal factor and deranged adrenal function.

I would like to discuss another aspect of adrenal function in *neoplastic disease*. Can we draw any conclusions about the function of the adrenal glands when these are stimulated by ACTH? (Dobriner *et al.*, 1950, 1951*a*, 1951*b*; Dobriner, 1951). In six normal males and nine patients with neoplastic disease, the ketosteroids and formaldehydogenic steroid levels were measured during a control period and during a period of 12 days when each of the subjects received 100 mg. of ACTH daily in four divided doses. In five normal subjects the increase of ketosteroids and formaldehydogenic steroids was remarkably similar (Table I). In one subject, no increase in ketosteroids was observed, but there was an increase of formaldehydogenic steroid to a level within the range of other normal males. Similar lack of response to ACTH as far as the ketosteroid excretion is concerned has been observed by us in several patients with neoplastic disease. It is clear then

I should like to show you some evidence concerning the causal relation of cancer and hormone production (Fig. 5). We collected the urine of a middle-aged woman during several periods in the course of more than three years (period I, II,

FIG. 5 Steroid excretion patterns of a patient with cancer of the breast before and after mastectomy.

and III) for the purpose of establishing steroid excretion patterns in her age group. Between period III and IV, cancer of the breast (grade 3) was diagnosed and a total mastectomy was performed. It can be seen that more than three years *before cancer was diagnosed* the abnormal steroid was present

metabolism and the status of adrenal function in neoplastic disease. In Fig. 6 the steroid excretion patterns of the most abundant urinary steroids before, during and after ACTH

Table II

EXCRETION OF KETOSTEROIDS AND FORMALDEHYDOGENIC STEROIDS BEFORE AND DURING ACTH INJECTIONS IN PATIENTS WITH NEOPLASTIC DISEASE

KS (*mg /24 hr*)		*FS* (*mg /24 hr*)	
Control	*12 Days ACTH*	*Control*	*12 Days ACTH*
14 7	63 6	4 2	11 9
16 6	42 7	1 7	37 8
17 0	41 7	5 4	24·0
24 7	32 6	1 0	18 3
16 5	38 6	2 4	25·5
10·7	48 6	—	—
16 0	60 8	10 5	40 4
15 1	55·0	0 2	11 9
17 1	74 9	5 0	43 0
Average			
16 5	50 9	3 8	26 6
Ratio			
1 0	3 1	1 0	7·0

Table III

COMPARISON OF EXCRETION OF KETOSTEROIDS AND FORMALDEHYDOGENIC STEROIDS BEFORE AND DURING ACTH INJECTIONS IN NORMAL SUBJECTS AND IN SUBJECTS WITH NEOPLASTIC DISEASE

	KS (*mg /24 hr*)		*FS* (*mg /24 hr*)	
	Control	*ACTH ST*	*Control*	*ACTH ST.*
Average				
Normal (6)	19 9	49 7	0 3	9 9
Neoplastic Disease (9)	16 5	50 9	3 8	26 6
Ratio				
Normal (6)	1 0	2·5	1 0	34 7
Neoplastic Disease (9)	1 0	3 1	1 0	7 0

that there is sometimes a changed or abnormal adrenal responsiveness to ACTH. If one compares the steroid excretion of the six normal subjects after ACTH with that of nine patients with neoplastic disease, one observes that the ketosteroid response of both groups is of the same order of

Table I

EXCRETION OF KETOSTEROIDS AND FORMALDEHYDOGENIC STEROIDS BEFORE AND DURING ACTH INJECTIONS IN NORMAL SUBJECTS

KS. (*mg /24 hr*)		*FS* (*mg /24 hr*)	
Control	*12 days ACTH*	*Control*	*12 days ACTH*
22 7	58 1	0·3	8 1
20·2	60·7	0 4	12·4
23 1	29 8	0·2	7·1
15·0	54 0	0 3	14 1
17 4	55 9	0 2	11·9
21 0	39·7	0 1	5·5
Average			
19 9	49 7	0·3	9·9
Ratio			
1·0	2 5	1·0	34·7

magnitude while, in contrast, there is a striking difference in the excretion of the formaldehydogenic steroids (Table II and III). During the control period in neoplastic patients, the formaldehydogenic steroid levels are higher than those in normal subjects. These findings indicate that the adrenals are already functioning at an increased level in the patients with neoplastic disease, and that their adrenals not only respond to ACTH but do so to a greater extent than the adrenals of normal subjects. This again indicates a different functional status of the adrenals in patients with neoplastic disease.

A study of the individual steroids excreted before and during stimulation of adrenal function by ACTH should give more detailed information about hormone production and

administration in patients with neoplasia is shown in comparison with the patterns of normal untreated subjects of the same age group. There was an increased excretion of both 11-oxygenated and 11-deoxysteroids during adrenal stimulation. It is important to note that this proves that the human adrenal produces at least two types of steroid hormones, one of the 11-oxygenated type like compound F (Kendall) and one of the 11-deoxy type, probably like compound S (Reichstein). On ACTH treatment the patients responded with different amounts of some steroids, and in addition new compounds appeared. These were not the same in all subjects. The results indicate qualitative and quantitative differences in the responsiveness of individual patients to adrenal stimulation.

We conclude from the evidence presented that both gonadal and adrenal functions are impaired in patients with neoplastic disease. There is evidence suggesting that the adrenal glands of these patients with neoplastic disease function differently both *qualitatively* and *quantitatively* from normal glands. There is a decrease in excretion of the metabolites of C-11 *deoxy hormones, and some metabolites of the C-11 oxygenated* steroids are qualitatively different from those of normal subjects. The excretion of C-11 oxygenated adrenal hormone metabolites is at a fairly normal or slightly increased level, but not at the maximum level to which the gland can be stimulated. One may speculate that not only an abnormal gland but also a changed pituitary adrenal regulation is present in neoplastic disease. The adrenal hormones play an important role in the course of the disease and may be involved in its cause.

I would like to express my deep appreciation to Dr. Thomas F. Gallagher for his generous and valuable assistance in the preparation of this manuscript.

This investigation was aided by grants from the American Cancer Society (on recommendation of the Committee on Growth of the

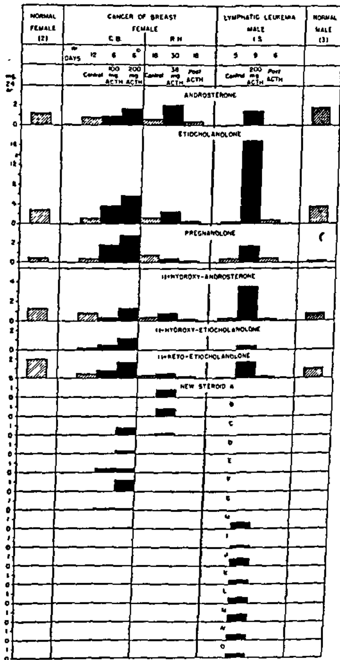


Fig. 6. Effect of ACTH administration on steroid excretion

adrenal metabolite, 11-hydroxyætiocholanolone, in a number of cancer patients before diagnosis of cancer indicates to me that there was already a metabolic change *before there was a cancer.*

FOLLEY: What about the possibility of excretion of steroids in the [illegible]

danger of some erroneous conclusions?

DOBRINER. We have done one experiment on normal humans. We injected a labelled steroid, and worked up the fæces, and we could discover only microgram amounts of metabolites, while more than 70 per cent of the injected material was found in the urine in the first day. Different species have different routes of steroid excretion. Mice and rats excrete steroids in the fæces for the most part. The horse is supposed to excrete very little in the fæces, and cows excrete steroids mostly in fæces.

SOMMERVILLE. Dr. Pearlman finds many progesterone metabolites in the bile of cattle, but not in that of pregnant women Have you used both cortisone acetate and cortisone, and do you find any difference in the urinary steroid pattern?

DOBRINER. We have only used cortisone acetate

SOMMERVILLE. I ask because when I administered pregnanediol to human subjects it reappeared in the urine, whereas administered pregnanediol diacetate did not do so.

DOBRINER· For metabolic studies we would like to have the free steroid, but since we could get only the acetate, we have studied that derivative.

BOYLAND Is there any evidence that response to ACTH is dependent on the saturation with ascorbic acid, or do all patients have plenty of ascorbic acid?

[illegible]

deficient diets There was considerable difficulty in getting the patients to take the diets, and when we got the riboflavin levels in the urine, it turned out that the diets weren't completely deficient.

[illegible]

a powerful leukæmogenic agent?

BURCHENAL: So many of the compounds which have an effect on cancer are also carcinogenic. I don't know whether it would be a leukæmogenic agent or not.

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DISCUSSION

DOBRINER: We do feel that we are justified in speaking of adrenal hormonal deficiency in neoplastic patients in the same way that we speak of a conditioned vitamin deficiency. The adrenals in these patients may be functioning well enough to supply the hormones required by a normal person, but in disease the tissues require larger amounts of adrenal hormones. Under conditions where the tissues require a larger amount of hormones, in this case adrenal hormones, there seems to be some mechanism which we don't understand, causing cessation of stimulation of the pituitary gland.

BEGG: There are really two aspects to this question: these changes in adrenal steroid patterns previous to the detection of the tumour may be one aspect, and another aspect the changes that take place in a host after the tumour is there.

DOBRINER: I was always very careful not to make a statement of what was primary and secondary. But certainly the occurrence of this

CHAIRMAN'S CLOSING REMARKS

You will agree it is difficult or perhaps impossible to summarize a meeting of this kind, so that the following are simply a few cursory remarks. I am sorry Astbury is not here this afternoon because I found his reaction to the meeting, especially the biological aspects of the subject, intriguing. I myself have never been an endocrinologist, but have always looked on the endocrine side of this field as certainly one of the most complex and the particular steroid field as the most complex sector of it. Astbury was very disturbed and anxious, listening to the biologists in particular, wondering how it was that they could deal with so involved a situation, when in experimentally varying one factor, and perhaps that rather inadequately known, they might also be varying a whole congeries of still other factors, quite unknown. On the other hand, I don't think we need feel too anxious : complexity is an inherent quality of the subject, and the field must be attacked subject to all these limitations.

If the best approach is that of planned experiment, it has also to be combined with a certain biological acumen. It is certain that Gardner's contribution was a very fine example of the planned experiment, with quite specific alterations of experimental procedure, everything being controlled as well as possible, and, as we now find, beginning to yield something which has meaning. As for biological acumen, we also find that beautifully illustrated, combined with the planned experiment, in Huggins' work, of which we had such a wonderful account this morning.

If I may say so, I think it would be quite unwise to adopt too physical or too rigid an approach [illegible]

the thing that crops up is very often some feature quite unexpected that the screen wasn't devised to detect, so that the

GARDNER: Did you notice any effect on alopecia and hair growth in your patients?

BURCHENAL: We have seen it in one patient, a girl treated with ACTH. She did not get a total alopecia as you see with the antifolics sometimes, but her hair definitely thinned out. However, on continued therapy, I think that wasn't so noticeable. I have not noticed it with the other ones. We have seen hirsutism in at least two children who were treated with cortisone. I don't think there was any other evidence of virilism.

BOYLAND: Does cortisone produce chromosome abnormalities? It would be interesting to see if it produces grey hair in dark mice.

DOBRINER: There are some experiments underway on that.

GARDNER. Baker has shown that if cortisone is topically applied it will prevent hair growth locally, and if systemically applied it will [illegible] same [illegible] rague [illegible] 'H.

HERTZ. Thorn found an increased pigmentation about the site of Compound E pellets.

BURCHENAL· One of our leukæmic patients, a boy of 16, developed a [illegible] see it often.

KELLIE. It is not very marked?

BURCHENAL· It depends. You don't push many patients to full Cushing's I think if you did you would get it more often.

BEGG: The Mayo people have included the production of striæ as part of their characteristics of the side effects of cortisone therapy.

CHAIRMAN'S CLOSING REMARKS

You will agree it is difficult or perhaps impossible to summarize a meeting of this kind, so that the following are simply a few cursory remarks. I am sorry Astbury is not here this afternoon because I found his reaction to the meeting, especially the biological aspects of the subject, intriguing. I myself have never been an endocrinologist, but have always looked on the endocrine side of this field as certainly one of the most complex and the particular steroid field as the most complex sector of it. Astbury was very disturbed and anxious, listening to the biologists in particular, wondering how it was that they could deal with so involved a situation, when in experimentally varying one factor, and perhaps that rather inadequately known, they might also be varying a whole congeries of still other factors, quite unknown. On the other hand, I don't think we need feel too anxious : complexity is an inherent quality of the subject, and the field must be attacked subject to all these limitations.

If the best approach is that of planned experiment, it has also to be combined with a certain biological acumen. It is certain that Gardner's contribution was a very fine example of the planned experiment, with quite specific alterations of experimental procedure, everything being controlled as well as possible, and, as we now find, beginning to yield something which has meaning. As for biological acumen, we also find that beautifully illustrated, combined with the planned experiment, in Huggins' work, of which we had such a wonderful account this morning.

If I may say so, I think it would be quite unwise to adopt too physical or too rigid an approach to the problem of screening. Certainly have the screen planned, thought out, designed as much as possible, but our experience is that, in spite of all, the thing that crops up is very often some feature quite unexpected that the screen wasn't devised to detect, so that the

factor of acumen or whatever you may care to call it is undoubtedly important.

I think we had at this meeting preliminary signs at any rate of some linking between this field and others, an essential process if the eventual picture is to be real. We separate them for mental convenience, but it is encouraging to feel that a link is beginning to appear between this field and, say, the general field of protein chemistry in relation to cancer. We had hoped Sir Robert Robinson might be here, bearing specially in mind his stimulating notion that the administration of a fresh steroid may result in the appearance of specific proteins.

Another matter of some interest is that the tumours which have responded in the chemotherapy results described by Dr. Burchenal are approximately the same as those which react to other chemotherapeutic procedures. We have been very puzzled to know why the lymphomata, plasmacytomas, reticuloses and so forth respond so briskly to the mustards, for instance, and why other malignant cell types don't so react. *One possibility is that these are the tumours derived* from the normal cell types particularly concerned with the production of protein in high amount, and many of them with the production of immune globulins and so on. We have wondered whether chemotherapeutic agents may have a selective action on these cells because they are possibly the seat of highly active specific protein synthesis in a way that other cells perhaps are not.

Apart from the questions of protein and protein anabolism and catabolism and so forth, in Hertz's work again we see interesting beginnings of a link-up with the general field of cell nutrition. I said at the time that Gardner's paper seemed to throw out a general principle, or possibility of such, in the significance of gonadotrophin in carcinogenesis. We made an interesting observation in this field about two years ago. Actually the fact was noted without our being aware of the proper circumstances for some time, but it appears that in the growth of two or three rapidly growing rat tumours there

is a most remarkable atrophy of the seminal vesicle. In the meantime, we have tried to determine what are the governing conditions, and it has been suggested that gonadotrophin may, in fact, be significant here. We wonder whether, as well as in carcinogenesis, a similar requirement may be important in the growth of the tumours themselves.

Then again Dobriner has just given us another example of work of great complexity and detail, but with two threads running through, that malignant disease may be associated either in its inception or course with decreased gonadal and adrenal function, and that the therapeutic action of ACTH and cortisone may possibly be based on specific adrenal stimulation of one kind or another.

Finally, we all felt that Foulds' account of the behaviour of the mammary tumours he described, being so susceptible, so dependent, and showing a most remarkable regression at the time of parturition and at the beginning of lactation, is a matter which, as I think he realizes, should be followed out very vigorously.

Those of us from this country are specially grateful for the massive American contribution to this meeting. In conclusion, I should like to convey my personal thanks, and thanks also on behalf of the Foundation, to all the speakers for the time and care which they have so generously devoted to the aims and success of the Colloquium.

BOOK II

STEROID HORMONES AND ENZYMES

At the time this colloquium was held, programme speakers were not asked to prepare or submit any manuscript. The editors are most grateful to the contributors for kindly providing *summaries* of their work, on which their remarks were based. The *general discussions* are, however, reproduced almost in full.

ASSAY, ACTIVITY AND PURIFICATION OF β-GLUCURONIDASE

W H. FISHMAN

I. Methods of Assay

THE hydrolysis of menthol glucuronide to menthol and glucuronic acid by tissue β-glucuronidase was first employed as the basis for glucuronidase assay by Masamune (1933). Measurements were made of the increase in the reducing power of the digest which is due to the liberated aldehyde group of glucuronic acid. At present the determination of reducing power is performed with a modification of the ceric sulphate reductimetric method of Miller and Van Slyke (1936). The disadvantages of this method are: (1) the necessity of using rather long periods of incubation; and (2) the residual reducing power of the tissue extract may be high in crude extracts. Nevertheless, the reductimetric method for determining β-glucuronidase activity has yielded valuable information in studies on the purification of the enzyme and on the determination of reaction kinetics.

The method of measuring β-glucuronidase activity employing phenolphthalein β-glucuronide (Talalay, Fishman, and Huggins, 1946; Fishman, Springer and Brunetti, 1948) is based on the principle that phenolphthalein in alkaline solution absorbs light at 540 millimicrons to an extent five hundred times that of the absorption of its glucuronide. Accordingly, measurements are made of the optical density of the alkalinized digests under standard conditions. The method is termed an *aglucuronometric* method, since the principle of the determination depends on the properties of the aglucurone radical. The same principle has been used by

Kerr (Kerr, Graham and Levvy, 1948) and Mills (1948) in their use of phenyl glucuronide as a substrate for β-glucuronidase. In this method, the liberated phenol was measured by means of the Folin-Ciocalteu reagent. Apparently the enzyme extract requires purification in order to reduce the value of the phenolic substances in the control digest. At the present time, the majority of investigators in this field employ phenolphthalein glucuronide as the substrate with which to assay glucuronidase activity.

II. Factors in Blood Which Influence β-Glucuronidase *Activity*

A. The addition of plasma to purified β-glucuronidase. It was found that the addition of plasma to purified dog liver β-glucuronidase in increasing amounts resulted in a progressive inhibition of the liver β-glucuronidase activity. This inhibitory activity is a property of a heat-stable, non-dialysable, protein-like component or components of the plasma. The inhibition appears to be a function of the enzyme concentration in the digest and does not influence the character of the *p*H-activity curve.

Studies have been done on plasma separated from the blood of human subjects with and without disease, in order to determine whether any characteristic increase or decrease of glucuronidase inhibitor was associated with the condition of the subject. In about 80 per cent of all subjects studied a progressive inhibition of purified β-glucuronidase is observed upon increasing the relative amount of plasma in the digest. Another 10 per cent of the patients did not show the presence of as much glucuronidase inhibitor as did the first group. In the remainder an interesting phenomenon was observed: at low concentration of the plasma in the digest, an activation rather than an inhibition of glucuronidase activity occurred, and with higher plasma concentration this effect may be reversed to one of inhibition. No explanation can be advanced for these findings at the present time.

B. Effect of dilution of the tissue homogenate on β-glucuronidase activity.

When one determines β-glucuronidase activity in different dilutions of the same homogenates of mouse tissues, essentially the same activity per gram of tissue is found. On the other hand, homogenates of rat and dog tissues often show higher activity per gram of tissue at low dilution of the homogenate. These findings emphasize the importance of working out systematically the best conditions for assay of β-glucuronidase in tissues of any given species. It was noted, also, that the tissues of the rat contained considerably more β-glucuronidase activity than did the tissues of the dog, mouse, or rabbit.

The observations described above made it clear that their adequate interpretation required a knowledge of the properties of pure β-glucuronidase. Once such a study had been made, the phenomena observed with crude extracts of liver glucuronidase could be reinvestigated.

III. Purification of β-Glucuronidase

The work which will now be described has been done in large part by Dr. Bernfeld in my laboratory. Many observations (Oshima, 1936; Karunairatnam and Levvy, 1949) indicated that a large variety of organic acids will inhibit the activity of β-glucuronidase. These include citric, malic, saccharic, gluconic, glucuronic, and ascorbic acids, as well as heparin. As a working hypothesis, we have assumed that β-glucuronidase in tissue may be combined with a number of acidic materials. Accordingly, in the purification procedure which has been developed, precipitation of the enzyme by ammonium sulphate in alkaline solution has been a new step which has been introduced. At the end of this purification procedure the preparation exhibits only one *p*H optimum at 4·5.

It has been reported by Mills (1948) and Levvy (Kerr, Graham and Levvy, 1948) that certain tissues contain several glucuronidases which differ only in their *p*H optima. When our best glucuronidase preparations are tested in the

presence of various acids such as adenylic, glucuronic, saccharic, and deoxyribonucleic acids separately, the pH optimum shifts to a higher value. Accordingly, it is suggested that the enzyme preparations of Mills and Levvy which show two peaks in the pH activity curve represent, not two different enzymes, but the same enzyme combined with one, two, or more different acidic compounds.

It was further observed that our purest β-glucuronidase preparation exhibited a marked activation of its activity in the presence of deoxyribonucleic acid. Accordingly, it is ... idase in tissue represents a complex of bel ... one of the most important the ... ie significance of this postu- of ... lation remains to be established through more experimental work.

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DISCUSSION

... dilution of ... ite the ... ctivity. ... on and ... te concentration ... enzyme activity?

Fishman. We have studied the effect of varying the sub... te concentration on the activity of glucuronidase. The conditions that we used in the dilution experiments were those constituting optimal ... ation and there is no doubt in my mind that the ... ite.

... relationship between β-glucuronidase ... on?

... relative activity of β-glucuronidase in ... te concentration, the curve ascends

to a maximum and then drops with excess substrate. This was found to be the case with three different substrates. Accordingly, the concentration of substrate selected in the enzyme assay corresponds with the optimal concentration observed in the substrate-activity curves.

FOLLEY: Have you tried to apply the equation of Lineweaver and Burk? They have evolved an equation which can be fitted to curves like yours, which show inhibition at high concentrations. From it two constants can be calculated, which would presumably correspond to the values you have found.

FISHMAN: We have not made those calculations.

MILLS: At what *p*H did you carry out the alkaline ammonium sulphate precipitation?

FISHMAN: At *p*H 8.

MILLS: We have observed progressive inactivation of glucuronidase at *p*H 8 and above. When you said that after alkaline precipitation you get only one electrophoretic component with isoelectric point at *p*H 7·5–8, I wondered whether you might be actually destroying preferentially one of the enzymes. About the shift in *p*H optimum which you find in the presence of saccharic acid and similar compounds, I think that can be explained, as I will try to show later, on the dif- [illegible]

[illegible]

magnesium?

FISHMAN: We haven't found any specific effects of the cations which would lead us to believe that one of these was essential for activity of the enzyme.

MILLS: We have tried various metals, calcium, zinc, magnesium, copper, and so on, as inhibitors and none of them had any significant effect.

ELSON: Have you tried any serum from cancer patients? You mention one carcinoma of the stomach from which there is apparently no dilution effect. That might indicate that in cancer patients there is probably an absence of inhibitors.

FISHMAN: These observations concern only tissue β-glucuronidase activity when determined in the cancerous and the surrounding tissue. This phenomenon where the tumour tissue enzyme activity is not affected by dilution, may or may not be related to the disease.

ELSON: I was wondering whether you have tried it in the blood plasma. You mentioned the plasma in surgical cases, where it doesn't

show this inhibitory activity. It might be that cancer patients in general don't show this.

FISHMAN: I don't believe that I can make a definite statement because you can observe all varieties of behaviour towards glucuronidase activity in the serum or plasma of cancer patients: inhibition, no change, and activation. We would like to obtain pure β-glucuronidase and test the effects on it of plasma, serum and tissue extracts. It is difficult to interpret the results with the relatively crude enzyme extract employed, because it may contain constituents which will influence the results.

STOREY: Does the glucuronidase prepared by alkaline ammonium sulphate precipitation behave in a homogeneous manner over a range of *p*H?

FISHMAN: As far as we know it does.

THE NATURE, PROPERTIES AND FUNCTION OF β-GLUCURONIDASE

G T. MILLS

A. The Preparation and Properties of β-Glucuronidase

During the past few years we have carried out work on the purification and properties of glucuronidase in order to learn more about its fundamental properties as an enzyme, before proceeding to a study of the physiological function of this enzyme. We have used spleen as the starting material for the preparation simply because it is the richest known source of glucuronidase, and we have used ox spleen since the material is readily available. The method used for the preparation of active enzyme extracts (Mills, 1948) is, very briefly, a defatting and dehydration of the tissue with acetone, followed by a water extraction and ammonium sulphate precipitation, with subsequent solution in water. The fractionation of these extracts has been carried out with ammonium sulphate using concentrations determined from salting out curves at various *p*H's (Mills, 1948). More recently we have been experimenting with low temperature acetone methods of fractionation, and these are yielding promising results.

Our main finding is that in ox spleen there appear to be three glucuronidase activities. These three main activities are distinguished by different *p*H optima, different affinities for various substrates and differing behaviours towards various inhibitors.

Some of the characteristics of these three enzymic activities are recorded in Table I.

The glucuronidases are inhibited by many mono- and dicarboxylic acids, the most potent of which is D-saccharic acid as first shown by Karunairatnam and Levvy (1949). These inhibitors affect the glucuronidase in different ways

Table I
PROPERTIES OF OX SPLEEN β-GLUCURONIDASE

Substrate	*Ox Spleen Glucuronidase*		
	I	II	III
pH optima (in acetate buffers)			
1-menthylglucuronide	4·5	5 0	3 4
phenylglucuronide	4 5	5 2	3 4
phenolphthalein glucuronide	4 5	5·2	3 4
Enzyme-substrates Dissociation constants at 38°C (calculated according to Lineweaver and Burk (1934))			
1-menthylglucuronide	$4\ 7 \times 10^{-3}$	$19\ 0 \times 10^{-3}$	
phenylglucuronide	$2{\cdot}0 \times 10^{-3}$	$5\ 1 \times 10^{-3}$	$1{\cdot}2 \times 10^{-3}$
phenolphthalein glucuronide	$0{\cdot}8 \times 10^{-3}$	$2\ 3 \times 10^{-3}$	$1{\cdot}0 \times 10^{-3}$
Energies of activation (calories/mol)			
phenylglucuronide	14,700	16,000	19,300
phenolphthalein glucuronide	14,400	16,700	18,900

and some of the findings on the type of inhibition exerted are shown in Table II.

Many other inhibitors are known for glucuronidase, for example, ascorbic acid, as first shown by Becker and Friedenwald (1949); this substance inhibits all three enzymes non-competitively. The poly-sulphonic acid trypanocidal

Table II
TYPE OF INHIBITION OF THE THREE OX SPLEEN β-GLUCURONIDASES EXERTED BY CERTAIN INHIBITORS

Inhibitor	*Enzyme*		
	I	II	III
Citrate	Competitive	No Inhibition	Competitive
Oxalate	*	No Inhibition	Competitive
Mucate	Competitive	Non-Competitive	Competitive
Saccharate	Competitive	Non-Competitive	Competitive

*Inhibition of such a low order as to prevent an accurate assessment of type.

drug Suramin inhibits glucuronidases at pH's below 4·5, and then shows no inhibition and even activation above pH 5·5. Heparin also inhibits the glucuronidases in a similar manner to Suramin.

From all the inhibition experiments it would appear that a prime requisite for a compound to inhibit glucuronidase is the possession of an acidic grouping in its molecule, which suggests that the active centre in the various glucuronidases may be basic in character.

An important question which arises concerning these three ox spleen glucuronidases is one which must be posed whenever an enzymic activity is fractionated into a number of apparently separate entities. Are we dealing with separate entities existing as such in tissues, do we modify one single enzyme in various ways during fractionation by the partial removal of inhibitory or activating substances, or do we start with a parent molecule and break it up into sub-units, each having distinctive properties? Evidence which bears on the problem includes the pH activity curves of an ox spleen extract at various substrate concentrations.

We have found that by carrying out such pH activity curves at different substrate concentrations in the range 0·00025 to 0·004 M phenolphthalein glucuronide, we obtain curves of widely differing shapes with optima at the various points which may be predicted from the behaviour of the purified fraction at various substrate concentrations, and we feel that this is good evidence that we are not simply producing artefacts by the fractionation procedures used.

Evidence from adsorption and electrophoretic studies of glucuronidase leads us to believe that glucuronidase exists as a complex of closely related proteins in the original tissue, possibly a series of protein molecules on a carrier protein molecule and that each activity can be separated, but is only active in the presence of the carrier molecule.

From our electrophoretic and other studies, we have concluded that the isoelectric points of the various glucuronidases all lie near to pH 5.

Table I

Properties of Ox Spleen β-Glucuronidase

Substrate	*Ox Spleen Glucuronidase*		
	I	II	III
pH optima (in acetate buffers)			
1-menthylglucuronide	4·5	5 0	3·4
phenylglucuronide	4 5	5·2	3·4
phenolphthalein glucuronide	4 5	5·2	3·4
Enzyme-substrates Dissociation constants at 38°C (calculated according to Lineweaver and Burk (1934))			
1-menthylglucuronide	$4{\cdot}7\times10^{-3}$	$19{\cdot}0\times10^{-3}$	
phenylglucuronide	$2{\cdot}0\times10^{-3}$	$5{\cdot}1\times10^{-3}$	$1{\cdot}2\times10^{-3}$
phenolphthalein glucuronide	$0{\cdot}8\times10^{-3}$	$2{\cdot}3\times10^{-3}$	$1\ 0\times10^{-3}$
Energies of activation (calories/mol)			
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Table II

Type of Inhibition of the Three Ox Spleen β-Glucuronidases Exerted by Certain Inhibitors

Inhibitor	*Enzyme*		
	I	II	III
Citrate	Competitive	No Inhibition	Competitive
Oxalate	*	No Inhibition	Competitive
Mucate	Competitive	Non-Competitive	Competitive
Saccharate	Competitive	Non-Competitive	Competitive

*Inhibition of such a low order as to prevent an accurate assessment of type.

level by 20 days of age, and is thereafter constant. From our results it is clear that the glucuronidase activity in the liver is constant when the liver is increasing at its most rapid rate, and this would not indicate any connection between glucuronidase activity of the liver and the growth rate of the liver. What this fundamental change in the glucuronidase content of the cell at 20 days of age means is not yet clear. It may be coincidental or not that at this age the animals are weaned, and the change in glucuronidase may be related to nutritional factors or to a change in the state of the endocrine balance of the animal.

We have found that the glucuronidase activities of the liver determined at the four *p*H values, 3·4, 4 5, 5·2, and 7·0, behave in an identical manner, and we take this to mean that all the glucuronidases of the liver behave as a group during *the growth and maturation of the animal. We have found* the same to apply in all other experiments we have carried out on the glucuronidase activity of the liver in various conditions.

We next turned to a study of rat liver during its phase of rapid growth which follows partial hepatectomy. Levvy *et al.* (1948) recorded that in mouse liver tissue following partial hepatectomy, there was an increase in glucuronidase activity 3–8 days after operation. In our experiments approximately 70 per cent of the liver of male albino rats was removed by the method of Higgins and Anderson (1931); the remaining 30 per cent rapidly regenerates, reaching 70 per cent of normal weight in 4 days and 100 per cent in 15 days.

Our results show that there is no change in liver glucuronidase activity during the first three or four days after operation, when liver weight, liver protein and liver DNA are increasing most rapidly, and the increase in glucuronidase activity only occurs when the rapid phase of growth is over.

This finding suggests that the increase in glucuronidase activity of rat liver which occurs 4 days after partial hepatectomy and which persists for up to 20 days, must be related to some other factor than proliferation. We have reached the

We have recently turned to a study of the glucuronidases of other tissues, particularly the liver. In this organ there definitely appears to be another glucuronidase, with optimal activity around pH 6·5–7·0. We had already gained the impression from kinetic examinations of ox spleen extracts, that such an enzyme existed in spleen, but in liver this activity at pH 6·5–7·0 is much more obvious. We are at present studying the separation of the liver glucuronidases by ammonium sulphate fractionation and low temperature acetone fractionation, and have obtained fractions containing increased amounts of this fourth glucuronidase.

In all experiments on the physiological function of glucuronidase we have assayed tissue extracts at four pH's, namely 3·4, 4·5, 5·2, and 7·0.

B. The Function of β-Glucuronidase

The function of glucuronidase in tissues is still a matter of conjecture, but various theories have been advanced concerning its mode of action. There is the metabolic role suggested by Dr. W. H. Fishman (Fishman, 1940, 1947; Fishman and Anlyan, 1947), who will no doubt say more about this theory, and I will therefore make no further reference to it. Levvy and his colleagues (Levvy, Kerr, and Campbell, 1948; Kerr, Campbell and Levvy, 1949, 1950) have suggested a relationship between the glucuronidase activity of mouse tissues and the extent of cellular proliferation occurring in those tissues.

Most of our experiments have been carried out on rats, and, in the first place, we found that the liver glucuronidase level of embryonic and young rats was less than that of adult animals—the adult activity being reached at about 30 days of age.

We have employed the deoxyribonucleic acid (DNA) content of the tissues as an indication of cell number, as suggested by Davidson and Leslie (1950 *a* and *b*), and this enables us to obtain an indication of the glucuronidase activity per cell. *Our results indicate that in the young rat the glucuronidase activity per cell is low at birth and rises to a constant*

when œstrogen is first secreted by the ovary in sufficient amount to affect this very sensitive target organ. It is possible that these results could be related to yours.

Mills: Yes, the change in our experiments may be related to the endocrine state of the animals.

Fishman. I would like to mention a difference which we have observed in the behaviour of our purified β-glucuronidase in the presence of citrate. The enzyme after alkaline ammonium sulphate is activated by citrate ions.

In the course of our purification we have compared at each step the ratio of hydrolysis of phenolphthalein glucuronic acid and of menthol glucuronic acid, and have observed a constant ratio in the course of purification. This agrees with your observations, emphasizing the fact that there is no actual qualitative difference in the ability of the various [illegible]

[illegible] of 6 4–6·5 [illegible]

Mills: There is one point about the citrate activation mentioned by Dr. Fishman. We observed rather a peculiar result when endeavouring to determine the type of inhibition exerted by oxalic acid. It inhibited the glucuronidase at a low substrate concentration; as the substrate concentration increased, inhibition decreased, and at higher substrate concentrations the effect of the oxalate was reversed and became very slight activation, the activation effects being not more than 10–15 per cent. It appeared to be a function of the substrate concentration.

Fison: Do these differences with age apply [illegible]

Fishman: The distribution of β-glucuronidase in mammalian tissue is very unusual, in that I have yet to find a tissue which did not contain some β-glucuronidase activity. This contrasts markedly with the

same conclusion from other experiments on liver damage caused by carbon tetrachloride which cannot be given in detail owing to lack of time. We have confirmed some of the experimental findings of Levvy and his colleagues on mice, but in view of our experiences with rats, we feel that the observed variations in liver glucuronidase activity must be related to some factor or factors other than cell proliferation, in all probability a similar process in both rats and mice.

An elucidation of the true function of β-glucuronidase will only be arrived at by the production of much more experimental evidence than we have at present available.

In conclusion I wish to thank Dr. J. Paul and Miss E. E. B. Smith for some of the results presented here.

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DISCUSSION

ON THE MECHANISM OF SYNTHESIS OF CONJUGATED GLUCURONIDES

I. D. E. STOREY

THE mechanism by which glucuronides are synthesized is of considerable interest in connection with the subject of this symposium, in view of the finding that certain of the steroid hormones and related compounds are excreted in this form by the animal body. It may be well to point out, however, that, besides its presence in these glucuronides of relatively low molecular weight, glucuronic acid is widely distributed throughout the body in mucopolysaccharides such as chondroitin sulphate and hyaluronic acid, and it is therefore a substance of great physiological importance.

In the early work on the origin of glucuronic acid and of glucuronides, in most cases the intact animal was used, and interpretation of the results is generally difficult. Suffice it [illegible]

[illegible] cursor. As such, he suggested lactate, or some other 3-carbon compound derived from the breakdown of glycogen. Similar conclusions were reached by Lipschitz and Bueding (1939) in their experiments with tissue slices. Using livers from fasted guinea-pigs, and borneol or menthol as glucuronidogenic agents, they were unable to demonstrate appreciable glucuronide formation from hexoses, glucosides or from glucuronic acid itself, but striking increases were obtained when lactate, pyruvate or dihydroxyacetone were added to the medium. Synthesis did not take place anaerobically, and cyanide, fluoride and iodoacetate all inhibited the process. From these results, they concluded that the synthesis was dependent upon oxidative processes as the source of energy, and that phosphorylations were involved.

situation for pepsin or trypsin, or even the acid and alkaline phosphatases. In order to construct a picture of the function of this enzyme, one must take into account the comparatively wide distribution of the enzyme in the tissue.

MEYER: Do you find a constant ratio of these four enzymes in different batches of spleen?

MILLS: No, with spleen we don't find very much of the *p*H 7 enzyme present at all. With the other enzymes we do find variations in the shape of the crude mixture *p*H activity curve. There do appear to be sometimes slight variations from one batch to another. They are not really fundamental changes. It may be just the way the spleens are treated before we get them.

MEYER: So the relationship between activities of each of those is constant from batch to batch?

MILLS: Relatively constant.

liver slices) it suppresses glucuronide synthesis at least 90 per cent.

Sulphate ion is also an inhibitor of the synthesis, and this has been shown to be due to the formation of an ester sulphate of o-aminophenol. It thus appears that the ester sulphate synthesizing system is competing with that forming glucuronide for the o-aminophenol available.

In the present work, the only substance which has been observed to cause any stimulation of the synthesis of glucuronides is bicarbonate. The rate of synthesis in phosphate Ringer is very low, but the addition of as little as 2·7 millimols /litre of bicarbonate can double the rate, and Krebs's bicarbonate Ringer, which contains about 16 millimols/litre at *p*H 7·3, raises it still further. This effect of bicarbonate is independent of the nutritional status of the animal, and is not due to changes in the *p*H of the medium, or to differences in respiratory rates in the two types of media. The most probable explanation appears to be that carbon dioxide fixation is in some way concerned in glucuronide synthesis. In an attempt to elucidate whether the Woods-Werkman reaction (carboxylation of pyruvate) or the carboxylation of α-ketoglutarate might be involved, experiments were performed involving the addition of certain components of the tricarboxylic acid cycle, such as succinate and malate, to slices in phosphate Ringer, but the results were inconclusive. The small increases sometimes observed could well be explained as being caused by respiratory carbon dioxide.

Finally, it might be of interest to mention the relationship between glucuronic acid and certain phases of pentose metabolism. In pentosurics, when a glucuronidogenic drug is administered, a small amount of glucuronic acid may be excreted, but there is also a greatly increased excretion of pentose (L-xylulose). Furthermore, Enklewitz and Lasker (1935) have shown that pentosurics give an increased excretion of pentose after administration of glucuronic acid. It would seem that these observations are well worthy of further investigation.

This *in vitro* work has been continued in the present investigations (Storey, 1950), but a simpler and more rapid method for the measurement of glucuronide synthesis, using o-aminophenol as aglycone, was employed (Levvy and Storey, 1949). With both fasted mice and guinea-pigs, considerable conjugation was observed even in the absence of the above-mentioned 3-carbon compounds, and addition of these to the medium at a concentration of 0·02 M was without effect. The reason for the discrepancy between these results and those of Lipschitz and Bueding is not at present apparent. Furthermore, the last-named authors used glucuronate at a concentration of only 0·005 M, whereas it has been found that at 0·02 M, it inhibits glucuronide synthesis 85 per cent, and at 0·01 M, 56 per cent. Gluconate and saccharate are also marked inhibitors, 26 per cent and 30 per cent respectively at the lower concentration, whereas all the other monocarboxylic and dicarboxylic acids tested showed only slight inhibitory activity. This apparently specific effect of glucuronate suggests that an analogy might be drawn with the catalysis by phosphorylases of glycosidic linkage formation, to form starch, glycogen and nucleosides, in all of which an aldose-1-phosphate is one component of the system. Cori, Cori and Green (1943) showed that glucose competitively inhibits the formation of glycogen by liver phosphorylase, and in the present instance it seems a reasonable explanation that glucuronate may be competing with glucuronic acid-1-phosphate, or some closely related compound.

Evidence has been obtained that glucuronide synthesis, like other biochemical synthetic processes, depends on high energy phosphate generated by oxidative metabolism. The observations of Lipschitz and Bueding (1939) on the inhibitory effect of cyanide have been confirmed, but their experiments with fluoride and iodoacetate are difficult to interpret. One agent which is known to suppress the formation of high energy phosphate is 2:4-dinitrophenol, and in the present work, at a concentration of 1×10^{-4} M (which may actually increase the rate of respiration of

investigate systems in which the aglycone is an alcohol which may not conjugate with sulphate.

STOREY: Regarding the relative rates of synthesis, the rates of synthesis which we have observed with *o*-aminophenol are approximately the same as those observed by Lipschutz and Bueding using

isolation.

FISHMAN. With regard
vitro, the relative failure
selves to observe incorpo

synthesis

MILLS: This is very interesting in
acid is
gates of
acid is
that the
It is quite possible that the synthetic system and conjugation system may be quite distinct, and that glucuronidase may play some part in

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DISCUSSION

[illegible] Referring to the work of Lipschitz and Bueding, it is of [illegible]

never been reported in nature.

I also found it difficult to interpret Lipschitz and Bueding's data because they made no distinction between the processes of glucuronic [illegible] and the [illegible] to [illegible] cu- [illegible]

glucuronate were more marked than those of saccharate, citrate and gluconate. We have just seen data that these substances are all inhibitors for the hydrolytic activity of β-glucuronidase in extracts, and we now see that they are inhibitors of synthesis. The failure to observe conjugation when pure glucuronic acid is present in the system may not be surprising. Lipschitz and [illegible] amount of [illegible] and perhaps there [illegible]

[illegible] er unusual [illegible] tive amine which may undergo reactions in the *in vitro* system, and I would think it would be important to isolate and identify aminophenylglucuronide after the slices have presumably synthesized a large quantity of this [illegible]

[illegible] ation and glucuronide [illegible] Sulphates and glucu- [illegible] We probably should

THE METABOLISM AND EXCRETION OF SYNTHETIC ŒSTROGENS, WITH SPECIAL REFERENCE TO THE FORMATION OF THE GLYCURONIDES

D. H. CURNOW and E. C. DODDS

The two most well-known mechanisms employed by the animal body for the detoxication and elimination of phenols are glycuronide and ethereal sulphate formation. It has long been the practice in carrying out experiments on the metabolism of phenols to measure increases in the total combined glucuronic acid excreted, or increases in the total ethereal sulphate, produced by feeding or injecting the phenol under study. In view of the large normal daily variation in the excretion of glucuronide in the urine, it is only possible to use these methods when large doses of phenols are administered. With the therapeutic dose of stilbœstrol, from a fraction of a milligram to 50 mg. daily, it is necessary to follow its conversion to glucuronide by some other method. Much larger doses, however, may be given to experimental animals, and the possibility of isolation of the metabolic products is greatly increased.

Although other workers have studied the conversion of some of the synthetic œstrogens to glucuronides, and Mazur and Schorr isolated stilbœstrol monoglucuronide from the urine of stilbœstrol-treated rabbits, it is proposed here to follow more closely the series of researches carried out at the Courtauld Institute of Biochemistry under the direction of Professor Dodds.

It had been shown that the urinary excretion of administered synthetic œstrogen in a biologically active state was much higher than in the case of administered natural steroid œstrogens.

STOREY: If you leave the magnesium out of the medium, it doesn't make much difference.

FOLLEY: Would it have the effect of decreasing the calcium ions? Have you investigated the effects of concentration of calcium on the synthesis?

STOREY: Calcium in the medium has no effect.

WILLIAMS-ASHMAN: Have you tried this work in homogenates?

methylene blue and brilliant cresyl blue on glucuronide synthesis? They also will uncouple oxidation from phosphorylation.

STOREY: I haven't done that. The dinitrophenol effect is rather nice and I thought the dyes would not penetrate.

WILLIAMS-ASHMAN: Methylene blue would.

STOREY: Also we are up against difficulty in the way of interference in the colour reaction. *ortho*-Aminophenol is not the ideal substance, but it gives a very rapid and very easy way of estimating activity. There is also the great advantage that you can estimate glucuronide directly instead of by the disappearance of your phenol.

sulphate and glucuronide formation. The ethereal sulphate is preferentially formed in a number of compounds, particularly ones which do not contain a phenol group; but with certain compounds, e.g., 4-aminodiphenyl, the aminostilbenes, and benzidine, the ethereal sulphate mechanism seems to fail, and they are excreted as glucuronides. We were struck by the fact that this process seems to be associated with increased toxicity and even with carcinogenicity, particularly in the case of the aminostilbenes.

*We have recently reported glucuronide synthesis by liver homogenates (Dutton, G. J., and Storey, I. D. E., 1951, *Biochem. J.*, 48, xxix). The product ... from that

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Stroud, at the Courtauld Institute, injected the synthetic œstrogens, hexœstrol, dienœstrol and stilbœstrol subcutaneously into rabbits, and studied the recovery in the urine of œstrogenically active material, by bioassay. His treatment of the urine consisted of a 20 hr. continuous extraction with benzene to obtain the "free" œstrogen, followed by 2 hours acid hydrolysis of the residual urine, and another 20 hr. benzene extraction to recover the "combined" œstrogen. In this way he obtained from 17–25 per cent of injected hexœstrol and stilbœstrol and 7 per cent of the injected dienœstrol. The reason for the low figure for dienœstrol will be seen later. Of the excreted œstrogen, some 70 per cent was found in the "free" fraction and 30 per cent "combined." These figures were obtained by *bioassay*, although some 5–15 per cent was recovered, after hydrolysis, as the crystalline hormone.

On the other hand, œstrogenic activity from the urine of *œstrone*-treated rabbits accounted for only 1·5 per cent of the activity of the injected hormone, 0·5 per cent "free" and 1 per cent "combined." From the urine of rabbits injected with 1·5 mg. of œstrone, 8 mg. of œstrone and 16 mg. of, probably, β-œstradiol were isolated. These experiments suggested a difference in the metabolic paths between the synthetic and the natural œstrogens.

To obtain more information on the metabolism of the synthetic œstrogens, Stroud next investigated the metabolism of some of the parent hydrocarbons of the œstrogens, and found that they were excreted as the corresponding phenols, e.g., diphenyl was converted to 4-hydroxydiphenyl in 25 per cent yield, stilbene to 4:4′-dihydroxystilbene in 3 per cent yield. No trace of the original hydrocarbons was found in the urine. The metabolism of diphenyl ether, which is itself not œstrogenic, is interesting in that it is excreted as 4-hydroxydiphenyl ether, which is an œstrogen, although a very weak one—100 mg. being required to produce vaginal œstrus in ovariectomized rats.

Continuing this type of approach, the metabolism of 4:4′-dimethoxydiphenyl ether and of 4-methoxydiphenyl was

investigated. In the rabbit the dimethoxydiphenyl ether was demethylated to 4-methoxy-4′-hydroxyphenyl ether and the mono-methoxydiphenyl to 4-hydroxydiphenyl; a little 4:4′-dihydroxydiphenyl was also produced.

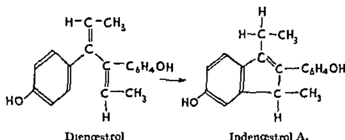
This work was followed up by Wilder Smith, Williams and Mrs. Simpson from a rather different point of view. In the treatment of cancer of the prostate with œstrogens the effective control of the carcinoma begins to fail after some time. It was thought that some adaptation by the body, involving changes in the metabolism of the œstrogen, may be responsible for the failure of œstrogen therapy.

The methods of study of urine from œstrogen-treated animals and man are, briefly, these: The urine is acidified with dilute HCl and extracted with ether. This ether extract contains the free phenols, and the glucuronides. These are separated by extracting the glucuronides as the sodium salts with saturated sodium bicarbonate. The phenols are then removed from the ether by NaOH extraction. The original urine containing any ethereal sulphate is hydrolysed and the liberated œstrogenic phenol extracted with ether. The three fractions—free phenol, glucuronide and sulphate—are then assayed in ovariectomized rats, or in the case of the glycuronide, may be assayed gravimetrically.

By this means it was shown that in rabbits injected with the synthetic œstrogen, up to 27 per cent of injected dienœstrol was eliminated as the glucuronide, 1–5 per cent appearing as the free phenol. With stilbœstrol the figure was sometimes as high as 46 per cent of the injected dose recovered as the glucuronide and up to 15 per cent as the free phenol.

In the cat, on the other hand, there was a much lower excretion—4 per cent recovery as glucuronide and 1 per cent as free œstrogen. The urine of the cat is acidic, while that of the rabbit is alkaline under normal conditions, but this difference was shown not to be responsible for the low excretion of glucuronide in the cat, in that producing acid rabbit urine by dietary means did not prevent the usual large excretion of œstrogen glucuronides.

In these experiments it was found that acid hydrolysis of urines containing dienœstrol always caused a loss of about 90 per cent of the original activity. This was traced to a facile cyclization of dienœstrol under acid conditions to produce the corresponding indene, identical with Hobday and Short's "*iso*dienœstrol" and Adler and Hagglund's "indenœstrol A."



Dienœstrol Indenœstrol A.

This compound possesses about one tenth of the activity of dienœstrol, and it is this which probably accounts for the low recoveries of dienœstrol obtained by Stroud in his early work.

But to return to Wilder Smith's work—very similar results to those obtained with *injected* œstrogens were obtained after the *oral* administration of hexœstrol, dienœstrol or stilbœstrol to rabbits.

Orally administered stilbœstrol glucuronide was excreted as the glucuronide in 13 per cent yield; subcutaneous injection produced a 25 per cent excretion of the same substance. This appears to show that either the glucuronide is not the end point of œstrogen metabolism or that the glucuronide is hydrolysed, some of the œstrogen takes other routes of metabolism and the usual 25 per cent or so is excreted again as the glucuronide.

In prostatic cancer patients, administered stilbœstrol is excreted in the urine as the glucuronide in yields of 10–60 per cent, and in normal women 10–75 per cent of administered is recovered as the glucuronide. In the prostate

patients up to 9 per cent was excreted as the ethereal sulphate, and in the normal women up to 5 per cent. In the rabbit there was a much lower excretion of sulphate, as little as 0·04–0·5 per cent of injected stilbœstrol, hexœstrol or dienœstrol appearing in this form. There is no evidence that prostatic cancer patients excrete excessively large amounts of the glucuronide, and some other factor must account for the failure of œstrogen therapy.

In the studies with rabbits, the monoglucuronides of stilbœstrol, hexœstrol and dienœstrol were isolated and identified and their chemical and biological properties were studied. They each have only 5–10 per cent of the œstrogenic activity of the free œstrogens, using either subcutaneous or intravaginal application in the bioassays.

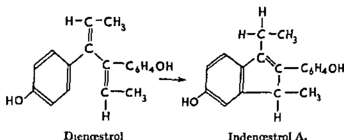
Recently we have started some work on the metabolism of 17-ethinylœstradiol. In the clinical use of this œstrogen there have been reports of sustained activity, and although these have not been very well supported, there has been suggested a stability in this compound not found with the unmodified natural œstrogens. Rabbits treated with ethinylœstradiol, however, do not excrete more than very small amounts of active material in the urine. This study is being pursued at the Courtauld Institute and should reveal some very interesting facts, especially with the use of a radioactive ethinylœstradiol.

In conclusion it is only necessary to re-emphasize the very important part that glycuronide synthesis plays in the metabolism and excretion of the synthetic œstrogens, and the ease of this synthesis and excretion, in contradistinction to the small excretion of the glycuronides of the natural steroid œstrogenic phenols.

DISCUSSION

[illegible]

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FOLLEY: I believe Emmenin, a preparation of œstriol glucuronide made in Montreal, is orally active. Does it give satisfactory results?

CURNOW: I don't know its activity relative to œstriol.

FOLLEY: Have you any evidence of the occurrence of glucuronides in clover or grass?

grass.

no acti

pared by Dr. Graham, which I tried to use for preganediol glucuronide in urine, but the hydrolysis was far from complete.

FISHMAN: Dr. Dobriner standardizes his hydrolysis with pregnanediol glucuronide and gauges the amount of enzyme to use from its

we have needed rather a lot of spleen to get enough enzyme.

MILLS Very roughly we get 3–5 mg. of phenylglucuronide hydro-

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practicable, as seems to have been done.

FISHMAN. It has the advantage that there are no tarry products produced in the course of enzyme hydrolysis, in contrast to the material resulting from acid hydrolysis.

STOREY: How long do you have to incubate the urine with glucuronidase?

FISHMAN: I think that Dobriner incubates overnight, about 24 hours

STOREY: I wondered whether the ether extraction of the urine before doing the quantitative determinations on glucuronides is really foolproof Some glucuronides are not soluble in ether, that's why we do them in acid solution

CURNOW. We have tested the glucuronides of the synthetic œstrogens by adding them to urine, and extracting with ether. They were quantitatively recovered.

FOLLEY: Do you get a monoglucuronide of stilbœstrol?

glucuronidase. Would it be possible to use vegetable glucuronidases, like baikalinase or ones produced by micro-organisms?

FISHMAN: Dr. Dobriner at the Cancer Memorial Hospital is using spleen glucuronidase added directly to urine concentrates after they've been fractionated, and he is satisfied with the results of hydrolysis, in that apparently there are no artefacts produced, no changes such as occur with acid hydrolysis. Dr. Doisy and his group in St. Louis are

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CURNOW: With injected stilbœstrol glucuronide, although there is about 75 per cent of it not excreted as such, it is interesting that during

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THE EFFECTS OF HORMONES ON β-GLUCURONIDASE ACTIVITY

W. H. FISHMAN

Mouse Tissues

In 1940 it was observed (Fishman, 1940) that the β-glucuronidase activity of liver, kidney, and spleen of animals fed menthol or borneol showed an increase which was not seen in the sex organs. Later it was found (Fishman and Fishman, 1944; Fishman, 1947*a*) that upon ovariectomy of female mice, the uterine β-glucuronidase activity exhibited a decrease and that this activity could be restored to normal levels by the administration of œstrogen. The enzyme activity of the non-sex organs, liver, kidney, and spleen, showed no significant change throughout these experiments. This effect seemed to be specific for œstrogens, and these observations have been confirmed and extended by ourselves and other investigators.

A fundamental question which presented itself for study was whether or not the increase in the glucuronidase activity of the œstrogen-stimulated uterus was the reflection merely of the growth of the tissue. When the data were examined, it was found that there was no correlation between the total uterine nitrogen and glucuronidase concentration. Since this study, we have encountered a number of situations in which marked growth of a tissue was not accompanied by an increase in its glucuronidase concentration; and conversely, tissues have been made to increase their glucuronidase activity markedly without any appreciable amount of cellular proliferation.

Several possibilities have been considered to explain the *in vivo* function of β-glucuronidase: the enzyme may be entirely hydrolytic, completely synthetic, both hydrolytic and

CURNOW: Yes.

FOLLEY: Why isn't it a diglucuronide? When one of the phenolic [illegible]

for example), and there may be some diglucuronide present, although this is doubtful.

MILLS: Phenolphthalein glucuronide is also a monoglucuronide.

McDonald and Odell (1947), who limited their investigation to the serum β-glucuronidase activity. It is interesting to note that the elevation in β-glucuronidase activity of the serum and plasma coincides with the excretion of progressively greater amounts of conjugated steroid glucuronides in the urine.

It has been observed that the postpartum fall in serum β-glucuronidase activity could be postponed by the administration of stilbœstrol (Fishman, Odell, Gill and Christensen, 1950). This would suggest that œstrogens may be responsible, in large part, for the elevated serum β-glucuronidase activity seen at pregnancy.

Odell and McDonald (1948) also demonstrated that women with the syndrome of pre-eclamptic toxæmia often possessed extraordinarily high serum β-glucuronidase values.

Observations on Human Vaginal Fluid β-Glucuronidase Activity

Odell and co-workers have reported (Odell and Burt, 1949) that high glucuronidase activity in the vaginal fluid was characteristic of women with cervical cancer, and this characteristic could be used as a diagnostic aid. In our studies (Fishman, Kasdon and Homburger, 1950) we have concentrated, first, on investigating the influence of physiological factors on vaginal fluid β-glucuronidase activity. It was observed that in normal subjects a very wide range of glucuronidase activity in vaginal fluid occurred, with low values predominating in the pre-menopausal state and high values frequently occurring in post-menopausal women. It was also observed that in women who have undergone pan-hysterectomy in which there remains no uterine tissue, high values of vaginal fluid β-glucuronidase activity were frequently found. From this control study it was concluded that the *uncritical* determination of vaginal fluid β-glucuronidase activity cannot be used as a diagnostic screening procedure.

synthetic, or have another function still unknown. The physiological experiments dealing with tissue changes in β-glucuronidase activity have been interpreted on the basis that β-glucuronidase participates predominently in the synthesis of glucuronides, a process of "metabolic conjugation" in which the conjugate represents the form in which the hormone is utilized in the tissue.

Interaction of β-Glucuronidase and Œstriol Glucuronide *in Vitro*

From an examination of the Michaelis Constants (K_m) and the affinities $\left(\frac{1}{K_m}\right)$ determined in experiments (Fishman, 1939; Talalay, Fishman and Huggins, 1946) in which the various glucuronides were hydrolysed by spleen β-glucuronidase, it is evident that the enzyme has a rather high affinity for œstriol glucuronide. No significant difference was found in the K_m values obtained when œstriol glucuronide was hydrolysed by mouse ovarian and splenic glucuronidase preparations.

Concerning the *in vivo* function of β-glucuronidase, it is desirable to point out that glucuronidase represents a protein of tissue which has a high affinity for œstriol glucuronide.

Blood β-Glucuronidase in Pregnancy and Toxæmias of Pregnancy

The activity of β-glucuronidase in the blood cells and plasma of women throughout pregnancy and parturition (Fishman, 1947*b*) has shown a relatively high concentration of the enzyme in the cells (which is true also of non-pregnant subjects) and a progressive increase in the glucuronidase which is terminated at parturition. This phenomenon has been investigated extensively and independently by

McDonald and Odell (1947), who limited their investigation to the serum β-glucuronidase activity. It is interesting to note that the elevation in β-glucuronidase activity of the serum and plasma coincides with the excretion of progressively greater amounts of conjugated steroid glucuronides in the urine.

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The level of β-glucuronidase activity in the vaginal fluid in menstruating women appears to be under the control of ovarian function. Thus, we have found a low range of values at mid-menstrual cycle. At the present time, work is under way in an attempt to further define the factors which control vaginal fluid β-glucuronidase activity, so that it may be possible to utilize this phenomenon for clinical purposes.

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DISCUSSION

FOLLEY: What do you understand exactly by vaginal secretion?

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of

FOLLEY: [illegible] cervical secretion.

FISHMAN: We know little concerning the glucuronidase activity of [illegible]

They produce [illegible] when you can pull it out in long threads, sometimes as much as one or two feet long, whereas at mid-cycle it is sticky and won't pull out in

the changes in glucuronidase

e placenta for glucuronidase

FISHMAN: I don't recall the figures which we obtained. They were not excessive for the human placenta.

GOUGH. Could glucuronidase activity in the uterine endometrium

FISHMAN: Very little is understood of the hormonal state immediately after parturition. There must be quite a violent transformation going on, and that is why we are not certain how to interpret the stilbœstrol factor. But this is one situation where œstrogen has an effect. Many people try to explain all of the behaviour of β-glucu-

endometrium of pregnant women?

FISHMAN: As I understand it, there is a decidual tissue which is not pure endometrium any more, because the growth of the placenta pre-

because we haven't been able to construct a satisfactory assay method. However, we have seen some very interesting phenomena in ascitic fluid or pleural fluid taken from patients with cancer. We usually

that there is an inhibitory effect of the fluid upon the cellular glucuronidase.

BUSH: Are there changes of glucuronidase activity with other hormones?

FISHMAN: Progesterone given to castrate female mice has no effect on uterine glucuronidase. Very little is known with respect to non-œstrogenic hormones.

GOUGH: Have you studied glucuronidase in an anovulatory cycle, when there is no pregnanediol and no temperature rise?

FISHMAN: No.

FOLLEY: Is the enzyme present in the urine?

FISHMAN: Yes.

WILLIAMS-ASHMAN: When you administer œstrogens to ovariectomized animals, how long do you have to administer them before you can observe any change in the uterine β-glucuronidase activity?

FISHMAN: That depends upon the mode of administration. The dosage was regulated to correspond exactly with the regular vaginal smear assay technique for œstrogens, so that they were given a divided dosage over three days and killed on the fifth day. But we did an experiment where a single injection of œstrogen dissolved in aqueous alcohol was given. The glucuronidase level was elevated about 20 per cent after one day, but by the third day it was down again.

There was one fact which may have some bearing on the time relationship of œstrogen action and β-glucuronidase response. The Astwood technique for assaying œstrogens involves the measurement of the increment in uterine weight of immature mice receiving œstrogen, and that increase in weight is due almost entirely to accumulation of water. Cell division, as I understand it, starts some time later. During this phase when water accumulates, the first six hours, there is no change in glucuronidase activity per gram of tissue, so that the β-glucuronidase response must occur after the phase in which water accumulates, and might be related to the cellular division in this instance.

ENZYMES IN THE CORPORA LUTEA OF THE RAT DURING PREGNANCY AND LACTATION*

R. K. MEYER

The activity of succinic dehydrogenase, malic dehydrogenase, adenosine triphosphatase, alkaline and acid phosphatases and anaerobic glycolysis enzymes was determined in homogenates of the corpora lutea of pregnant and lactating rats. All enzymes varied in activity in both reproductive phases. Especially noteworthy were the increases in succinic dehydrogenase, malic dehydrogenase and anaerobic glycolysis during the first eleven days of pregnancy. The maximum values for these enzymes were found during the time that the corpora of pregnancy were attaining their maximum weight.

Acid phosphatase and adenosine triphosphatase declined in activity in the corpora of pregnancy during the first eleven and fifteen days, respectively. The greatest activity for the enzymes was observed during the last six days of pregnancy.

Anaerobic glycolysis, acid phosphatase and adenosine triphosphatase exhibited relatively little change in levels of activity during pregnancy, when compared with malic and succinic dehydrogenase. However, the activity of adenosine triphosphatase and alkaline phosphatase in the degenerating corpora of pregnancy was much greater than that found when the corpora were functional. The activity of the other enzymes in the degenerating corpora of pregnancy was decreased markedly soon after parturition.

Histochemical studies showed that alkaline phosphatase in the corpora of both pregnancy and lactation is largely concentrated in the vascular tissue, with relatively little, if any,

*The data presented in this series of reports are the results of the cooperative efforts of my colleague, Dr. W H McShan, our graduate students, and Dr E. G. Shipley

in the lutein tissue. This observation serves to illustrate the importance of histochemical studies for the localization of enzymes in specific cells, and in interpreting data obtained by biochemical determinations of enzyme activities of an organ.

The succinic dehydrogenase activity of the corpora lutea of pregnant rats hypophysectomized on the eighth day of gestation and killed on the twelfth day is decreased when compared with that of corpora of normal controls in the same stage of pregnancy. However, the enzyme activity in the corpora of the rats hypophysectomized on the twelfth day of pregnancy and killed on the fifteenth day, is like that found in control rats.

The data suggest that for the first eight to ten days of gestation in the rat the activity of succinic dehydrogenase of the corpora is dependent on the pituitary gland, and that after the tenth day the placenta is largely, if not wholly, responsible for maintaining the activity.

During lactation the succinic dehydrogenase activity of the corpora decreases markedly after hypophysectomy on the fourth, eighth or twelfth days, which demonstrates the importance of the pituitary gland in the maintenance of the function of this enzyme during lactation.

Definite patterns of changes in enzyme activity occur in the corpora lutea of the rat under the natural and experimental conditions studied. These patterns are correlated with anatomical and functional changes in the gland, and with the level of trophic hormones acting on the gland.

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of involution which follows weaning there is a fall in the enzyme concentration. I wonder if the enzyme content of both these tissues is under

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MEYER: It is certainly true that the correlations are there. With regard to the action of luteotrophin, during lactation when we have this large increase in alkaline phosphatase, larger than during pregnancy, the corpora are small. At the same time the mammary glands are, of course, very active, so that makes a good correlation. It may be that during lactation the output of luteotrophin from the pituitary

during pregnancy and the absence during lactation. The succinic dehydrogenase and alkaline phosphatase are high, for example, in these small corpora lutea during lactation. In pregnancy the corpora will increase 400 per cent in size. We have not been able to explain what the "survival value" is of this large corpus luteum. It may be that during the latter half of pregnancy in the rat the corpus luteum is acting as a supplementary producer of adrenal cortical hormones, because one can at least build up a case that the metabolic stress during the latter half of pregnancy is tremendous and the corpus luteum may not be producing much progesterone at that time. This is indicated by the studies of Atkinson and Hooker on the mouse. As we know, progesterone will maintain pregnancy in the adrenalectomized animal, and the corpora lutea are sufficient during pregnancy to maintain life in the adrenalectomized rat, so there is undoubtedly an interplay between these two tissues.

pregnancy.

MEYER: Everett's work shows the importance of studying cholesterol content during these functional phases, and that deserves further study.

KOCHAKIAN: There is a general trend towards the view that alkaline phosphatase is involved in reorganization ("reshuffling"), breakdown and build-up of proteins rather than the splitting of phosphate. I think your thoughts fit right in with this concept

FISHMAN It is certainly easier to imagine that these changes in enzyme concentration are associated with the synthesis of cell material rather than with their breakdown, however we may measure the activities *in vitro*. I was wondering what might happen to an

ENZYMES IN THE PLACENTOMA OF THE RAT

R. K. MEYER

Placentomata produced artificially in the pseudopregnant rat were examined for the presence and quantities of alkaline and acid phosphatases, β-glucuronidase, succinic and malic dehydrogenases, and nucleic acids. Tissue was collected for analyses on the sixth, seventh, ninth, tenth, eleventh and twelfth days after the uterus was traumatized.

Alkaline phosphatase activity increases rapidly up to the seventh day after the uterus has been traumatized. At this time the formative stage of the placentomata has been reached. During the involutional and degenerative phases (ninth to twelfth days) the alkaline phosphatase declines to very low levels. Acid phosphatase, however, is low when the placentomata are developing, reaching high values at the time necrosis is most marked.

Both nucleic acids are greatest in concentration on the seventh day, and decline slowly through day eleven.

Succinic and malic dehydrogenase and β-glucuronidase are found most active during involution and necrosis.

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DISCUSSION

... alkaline phosphatase in mammary

TICKNER Can you tell me at what stage of pregnancy the adrenals were removed?

MEYER· The adrenals were removed on the 12th day.

BUSH. How did you measure the progesterone?

MEYER The data are from Laqueur and Koets They didn't measure progesterone directly. They computed it from the ketosteroids.

BUSH Right in the corpora lutea?

MEYER: Yes. They also determined the total lipid and cholesterol content and water content in the corpora of pregnancy.

FOLLEY: Have you studied the adrenal cortex?

MEYER. No, we haven't.

FOLLEY: We have studied the alkaline phosphatase in the whole kidney—we've not attempted to differentiate between the cortex and medulla—and we find that the alkaline phosphatase level in the kidney is surprisingly constant in pregnancy and lactation.

KOCHAKIAN: I would expect as much from œstrogen studies.

FOLLEY· Is your alkaline phosphatase fully activated with magnesium? Have you worked out the percentage activation that can be brought about by magnesium to see whether there are any differences there?

MEYER. I think that we have worked out the optimum conditions, though I can't remember the exact data. This was done three years ago.

KOCHAKIAN Has anybody actually shown that you can get a difference using full activation?

FOLLEY I don't think anyone has shown that, but differences in the percentage activation of the homogenates have been observed, though these were not sufficiently great so that different trends were observed at full activation from those shown by the enzyme in its natural state.

KOCHAKIAN: We have some phosphatase studies, with and without magnesium, and no divergence in results has been observed as yet.

greater activity than has been demonstrated. Furthermore, in these homogenates we have to remember that we have disrupted the cytoarchitecture of the cell and that the enzymes may not be working at the potential activity which they would have in the intact cell.

adrenalectomized pregnant rat. Will the animal's corpus luteum protect it?

MEYER: We have been doing some experiments along that line. If you adrenalectomize pregnant rats the pregnancy will be maintained, although parturition is difficult. The corpora lutea do not undergo [illegible] parent animal, [illegible] ophy to 7 and 8 [illegible] This indicates [illegible] as an end organ.

FOLLEY: Have you done any experiments on making rats pseudo-pregnant and then giving lactogenic hormone? Might it put up the alkaline phosphatase, for instance, above the value you get in late pregnancy?

[illegible] on it.

[illegible] those.

MEYER: Yes. We made similar studies, but not as completely, with succinic dehydrogenase and adenosinetriphosphatase during pseudo-pregnancy in rabbits. We saw much the same picture.

[illegible] possibilities.

MEYER: We haven't been doing anything with lutein tissue. It's on the programme, but we were, so to speak, cutting our teeth on liver.

THE EFFECTS ON ENZYMES OF ANDROGENS AND GROWTH HORMONE*

CHARLES D. KOCHAKIAN

Dr. Kochakian presented data from many experiments by himself and co-workers. Most of this material has been published elsewhere, and summaries of these publications, prepared by Dr. Kochakian, are given below.

THE EFFECT OF CASTRATION AND TESTOSTERONE PROPIONATE ON D-AMINO ACID OXIDASE ACTIVITY IN THE MOUSE. *Science* 98, 89 (1943). L. C. Clark, Jr., C. D. Kochakian and R. Phyllis Fox.

THE mouse kidney loses part of its ability to oxidatively deaminate D-alanine as a result of castration. The administration of testosterone propionate not only restores this property, but increases it above normal.

THE EFFECT OF CASTRATION AND TESTOSTERONE PROPIONATE ON THE "ALKALINE" AND "ACID" PHOSPHATASES OF THE KIDNEY, LIVER, AND INTESTINES OF THE MOUSE. *J. biol. Chem.*, 153, 669-674 (1944) Charles D. Kochakian and R. Phyllis Fox

There was a decrease in the "alkaline" (pH 9 8) phosphatase accompanied by an increase in the "acid" phosphatase (pH 4·9) in the kidneys of normal and castrated mice treated for 35 and 115 days with a subcutaneous pellet of testosterone propionate. Castration resulted in a decrease in both of the enzymes in about the same proportion as the diminution in kidney weight.

The enzymes of the liver and intestine were not significantly changed as a result of testosterone propionate treatment or castration.

*The work reported in th [illegible]

[illegible]

maintain pregnancy depend in any way on the size of the litter? Supposing you happened to have a small litter or took some away, would one corpus luteum be enough?

MEYER: I don't know. You mean that if you do a unilateral hysterectomy during pregnancy, the number of corpora lutea needed would be less than one? That we haven't tried.

BUSH: How does the activity of ADT-ase compare with that of muscle?

MEYER: It is low.

3β: 17α-diol caused decreases of 33 to 39 per cent. The changes were not related to changes in kidney weight or amount of material absorbed.

The increases in arginase activity represented greater amounts of enzyme and not a production of arginase activators.

The kidneys of the castrated mice contained the same amount of total arginase but greater amounts per gram of tissue than did those of the normal mice.

THE EFFECT OF DOSE AND NUTRITIVE STATE ON KIDNEY ARGINASE AFTER STEROID STIMULATION. *J. biol. Chem.*, 161, 115-125 (1945)
Charles D. Kochakian

The increase in arginase activity obtained in the kidneys of castrated mice treated for 30 days with various steroids implanted subcutaneously as pellets is related to the amount and the chemical structure of the compound absorbed. There is at first a decrease in arginase activity of the kidney which occurs during the phase when the kidney is increasing in size to its maximum response. This initial phase is followed by a rapid increase in arginase activity. These two phases are altered by the chemical structure of the steroid. Testosterone, testosterone propionate, androstane-3α:17α-diol, and androstan-17α-ol-3-one produce almost identical responses per mol of steroid absorbed. The introduction of the 17-methyl group (17-methyltestosterone and 17-methyl-androstane-3α· 17α-diol) causes a rapid initial increase instead of a decrease in arginase activity, which then continues at a slower rate of increase until it becomes identical with that of the former compounds.

Undernutrition does not affect the ability of the steroids to stimulate arginase activity per gram of tissue, but decreases the total increase because of the smaller increase in kidney size.

It is suggested that the increased arginase activity is related to synthetic processes such as protein anabolism and glycocyamine formation.

The tissues of the older mice contained more "alkaline" phosphatase than those of the younger animals.

HISTOCHEMICAL STUDY OF "ALKALINE" PHOSPATASE OF THE KIDNEY OF THE CASTRATED MOUSE AFTER STIMULATION WITH VARIOUS ANDROGENS. *Amer. J. Physiol*, 152, 257-262 (1948) Charles D Kochakian.

The distribution of the "alkaline" phosphatase of the kidney of the mouse has been studied after treatment with eighteen different steroids, many of which were studied at several dose levels. There was a progressive decrease in the enzyme from the distal end of the proximal convoluted tubule towards the glomerulus, with a slight increase in concentration at the glomerular end. These changes paralleled the increase in kidney size under steroid stimulation, so that at maximum stimulation the kidney showed varying degrees of depletion of the enzyme in its nephrons.

THE EFFECT OF CASTRATION AND VARIOUS STEROIDS ON THE ARGINASE ACTIVITY OF THE TISSUES OF THE MOUSE. *J. biol. Chem*, 155, 579-589 (1944) Charles D Kochakian.

Mice weighing 16 5 to 19·5 g. were castrated, and one month later 14$\pm$1 mg. pellets of various steroids were implanted subcutaneously. Arginase determinations were made 10 and 30 days later. None of the steroids affected the enzyme content of the liver or intestine, but many of these compounds markedly increased and a few decreased the arginase content of the kidneys. The order of change in per cent difference per gram of kidney tissue for the 30 day experiments was as follows: methyltestosterone, 632; testosterone, 584; testosterone propionate, 308; 17-methyl-androstane-3α:17α-diol, 269; androstan-17α-ol-3-one, 135; α-œstradiol, 88; androstane-3α:17α-diol, 71; 17-vinyltestosterone, 55; testosterone-3-acetate-17-propionate, 35. Much greater changes were obtained when the values were calculated on the basis of total tissue. Eighteen other compounds had no effect, and *iso*androsterone, 17-methylandrostene-3β· 17α-diol, and 17-methylandrostane-

3β: 17α-diol caused decreases of 33 to 39 per cent. The changes were not related to changes in kidney weight or amount of material absorbed.

The increases in arginase activity represented greater amounts of enzyme and not a production of arginase activators.

The kidneys of the castrated mice contained the same amount of total arginase but greater amounts per gram of tissue than did those of the normal mice.

THE EFFECT OF DOSE AND NUTRITIVE STATE ON KIDNEY ARGINASE AFTER STEROID STIMULATION. *J. biol Chem*, **161**, 115-125 (1945) Charles D. Kochakian.

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Undernutrition does not affect the ability of the steroids to stimulate arginase activity per gram of tissue, but decreases the total increase because of the smaller increase in kidney size.

It is suggested that the increased arginase activity is related to synthetic processes such as protein anabolism and glycocyamine formation.

EFFECT OF TESTOSTERONE PROPIONATE AND GROWTH HORMONE ON THE ARGINASE AND PHOSPHATASES OF THE ORGANS OF THE MOUSE. *Amer. J. Physiol.*, 155, 262-264 (1948) Charles D. Kochakian and Constance E. Stettner.

Mice were castrated at 17- to 19-grams body weight and one month later were (1) implanted subcutaneously with a 14–15 mg. pellet of testosterone propionate, (2) injected subcutaneously with 1·1 rat U/day of growth hormone, and (3) treated simultaneously with both hormones for 10-, 20- and 34-day periods. Testosterone propionate produced the expected marked increase in kidney arginase, small increase in "acid" (*p*H 5·4) phosphatase and marked decrease in "alkaline" (*p*H 9·8) phosphatase activities. Growth hormone was ineffective, but when administered simultaneously with testosterone propionate, it decreased the arginase-stimulating effect of the androgen to one-half.

The liver enzymes were not affected by either of the hormones. The small increases in liver size were accompanied by proportionate increases in the enzyme activities.

THE EFFECT OF ANDROGENS AND HYPOPHYSECTOMY ON ARGINASE AND PHOSPHATASES OF THE KIDNEY AND LIVER OF THE RAT. *Arch. Biochem.*, 29, 114-123 (1950) Charles D. Kochakian and Evangeline Robertson.

Androgens produced a small increase in weight and an increase in proportion to dose in arginase activity of the kidney of the castrated rat. The "alkaline" phosphatase was slightly increased and the "acid" phosphatase changed in proportion to the weight. The arginase activity of the liver was not affected by the androgens, but the alkaline phosphatase showed an irregular but persistent small increase.

Hypophysectomy of adult male rats resulted in a marked decrease in arginase, a slight decrease in acid phosphatase, and an increase in alkaline phosphatase activity of the liver. All of these enzymes of the kidney rapidly decreased. Testosterone did not affect the liver enzymes but restored the arginase and alkaline phosphatase activities of the kidney.

EFFECT OF CASTRATION AND ANDROGENS ON BODY AND ORGAN WEIGHTS, AND THE ARGINASE AND PHOSPHATASES OF KIDNEY AND LIVER OF THE MALE SYRIAN HAMSTER. *Amer. J. Physiol.*, 153, 210-214 (1948) Charles D. Kochakian, Mary N. Bartlett and José Gongora.

Castration caused a decrease in the size of the seminal vesicles and prostates but no change in the kidney or liver. The administration of testosterone propionate by injection or by the subcutaneous implantation of a pellet for 20 and 140 days increased the seminal vesicles and prostates of castrated hamsters to greater than normal but did not affect the size of the kidney or liver. Pellets of testosterone and 17-methyltestosterone implanted subcutaneously for 20 days produced similar responses.

The arginase of the kidney increased as a result of castration and decreased to normal with the various androgen treatments. The "alkaline" phosphatase, on the other hand, decreased after castration and was restored to normal with androgen treatment. The "acid" phosphatase of the kidney and the arginase and phosphatases of the liver were not affected by castration or by the androgen treatment.

EFFECT OF CASTRATION AND STEROIDS ON THE ARGINASE AND PHOSPHATASES OF THE ORGANS OF THE GUINEA PIG *Amer. J. Physiol*, 155, 251-254 (1948) Jane Harrison Humm, Charles D Kochakian and Mary N. Bartlett.

Male guineapigs were castrated at about 250 g. body weight. Thirty-five days later they were implanted subcutaneously with pellets of the following steroids: 17-methyltestosterone; testosterone; testosterone propionate; 17-methylandrostan-17α-ol-3-one; androstan-17α-ol-3-one; 17-methylandrostane-3α:17α-diol; and androstane-3α·17α-diol. The dose of steroid was varied by the number of pellets implanted. Castration produced a decrease in the arginase activities of the kidney after 60 days, but not after 120 days. None of the steroids produced any remarkable changes. The greatest increase, 79 per cent, was produced by 17-methyltestosterone, while testosterone was completely ineffective. The administration of a relatively large dose, 12.5 mg./day, of testos-

terone propionate for 14 days produced only a 38 per cent increase. Castration produced a decrease in the "alkaline" phosphatase of the kidney, which was restored toward normal by the various steroids. None of the enzymes of the liver or the "acid" phosphatase of the kidney were affected by castration or the steroids.

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DISCUSSION

FOLLEY: I was very interested in your theory of the possible function of arginase in the kidney. That is something of a mystery, of course. Have you done any experiments designed to get actual evidence for the transfer of the amino group to glycocyamine?

KOCHAKIAN: We planned it, but other things have been more pressing.

FOLLEY:

KOCHAKI

attracted b

YOUNG.

You compared, for instance, hypophysectomized animals and normal animals.

KOCHAKIAN. The nitrogen metabolism was determined on those animals. In the hypophysectomized animals food intake will go down, while the castrated animals will maintain body weight and nitrogen . The hypophysec- g. per day over a veight and nitrogen equilibrium.

YOUNG: Your first experiment was made on groups of animals which were not consuming the same amounts of food?

KOCHAKIAN: That's right.

YOUNG: Do you find no change in blood amino-acid with growth hormone?

KOCHAKIAN: I haven't done it with growth hormone; just with testosterone.

FOLLEY: Is there any effect of androgens *in vitro* on arginase and phosphatase?

KOCHAKIAN: We did add testosterone to homogenate and got nothing Also we did some experiments with adrenal cortex extract and got nothing.

GREENBAUM: In your experiments with androgen you got a very

questioning whether it has anything to do with urea formation in the liver.

STOREY: I think it is a very interesting suggestion, and it does raise the possibility that some of these enzymes may have a function other than the rather obvious one that we have always concluded that they have. I was thinking too of the suggestion that was thrown out yesterday about glucuronidase

KOCHAKIAN: The work that I am going to talk about this afternoon on the adrenal cortex suggests that arginase hasn't anything to do with urea formation· it is probably more concerned with internal reshuffling of protein.

FOLLEY. What do you think of Edlbacher's theory of the function of arginase?

KOCHAKIAN: That was the hypothesis which first gave me some support in these views. I gather, however, that Fraenkel-Conrat thinks that this is a specialized type of synthesis.

FOLLEY: We've done a lot of studies on the mammary gland, some of which Dr. Greenbaum will mention this afternoon, and we are faced with the same problem there—finding a function for the mammary gland arginase Dr Greenbaum rather clings to the classical idea that the mammary arginase is concerned in the formation of urea. However, there are difficulties to be faced, since in preliminary experiments he hasn't been able to observe urea formation in mammary gland slices in the rat. Another difficulty is that in herbivores, particularly ruminants, the mammary gland arginase level is very low. Arginase doesn't seem to play a very important part in lactation in herbivores We find the same species differences in the liver arginase levels. Rat and mouse liver have a much higher content of arginase than the liver of the rabbit, goat, or cow. Have you any observations on this?

KOCHAKIAN: As I remember, our guinea pigs showed low arginase activity.

FOLLEY: We haven't [illegible]

[illegible] meat diet slightly lowered the liver arginase levels.

KOCHAKIAN: How long did you feed it?

FOLLEY: Three weeks.

KOCHAKIAN: We have [illegible] a source of prot[illegible] on arginase. If [illegible] you get a possible slight increase, but after about three weeks you do get a definite increase. Lightbody also found that same effect several years ago.

FOLLEY: Dr. Greenbaum, you ran some experiments with an ordinary high protein diet, didn't you?

GREENBAUM: Yes. Our results didn't really confirm Dr. Lightbody's finding. We did get an increased [illegible] 7 days on a [illegible] another 10 [illegible] hat an animal which has been chewing up protein at a high level for so long is going to have a very increased arginase.

KOCHAKIAN: That's right. And that's what makes me even more suspicious of the urea function for arginase.

COMPARISON OF β-GLUCURONIDASE ACTIVITY IN TISSUE OF FŒTAL, NEW-BORN, AND INFANT ANIMALS WITH THOSE OF THE MOTHER (MOUSE, DOG, AND HUMAN)

W. H. FISHMAN

It has been reported by Dr. Levvy and his co-workers in Edinburgh* that the activity of β-glucuronidase in a tissue was correlated with the state of cellular proliferation and growth. Thus, for example, the tissues of fœtal, new-born, and infant mice exhibited high glucuronidase activity when compared to the corresponding tissues of the mother. These investigators employed phenyl glucuronide as substrate and subjected the homogenate to incubation at an acid *p*H for thirty minutes, followed by an ammonium sulphate precipitation procedure, before enzyme assay.

In repeating these experiments, we prepared fresh tissue homogenates which were centrifuged at high speed and the supernatants were assayed for β-glucuronidase activity, employing phenolphthalein β-glucuronide as substrate.

The enzyme activity of the kidney, spleen, brain, stomach, and heart of fœtal mice and one-, two-, and three-week-old litter-mates, never showed an increased activity as compared with the organs of the maternal organism. Similar results were found in the dog, comparing fœtal and maternal tissue. Human embryonic tissue exhibited less glucuronidase activity than had been found in organs of adult humans. It would appear that our findings are at variance with those of Dr. Levvy, and we have suggested to him that the discrepancy may lie either in the different experimental techniques which our two laboratories employ or in strain differences.

*Levvy, G. A., Kerr, L. M. H., and Campbell, J. G. (1948) *Biochem. J.* 42, 462

DISCUSSION

MILLS: Our experience has been not unlike that of Dr. Fishman. The levels of liver glucuronidase activity in embryonic or young rats (birth to 3 weeks) are less than in adults. We have used an extraction procedure similar to that of Levvy, using phenyl and phenolphthalein glucuronide for assay, and also a technique like that of Fishman, and the results are very similar. In the case of mice, we have obtained results very similar to those of Levvy—namely a higher liver glucuronidase activity in young mice than in adult, and we feel that the difference between rats and mice is a species difference.

FISHMAN: As I said yesterday, I feel that on the basis of the glucuronidase concentration to uterine nitrogen relationship, that the amount of protoplasm *per se* is not the important factor, and I think Dr. Mills is chiefly of that opinion. It would look more as if there is some more

to growth changes alone.

RELATION OF GLUCURONIDASE TO ACTION OF GONADAL HORMONES

R. K. MEYER

The β-glucuronidase activity of the livers of mice, rats and frogs receiving α-œstradiol or diethylstilbœstrol is not significantly higher than that of livers of control animals.

Doses of œstrogen which cause pituitary hypertrophy in ovariectomized mice and hypertrophy of the seminal vesicles in castrated rats did not elevate the glucuronidase activity in these tissues.

When graded doses of diethylstilbœstrol are given to ovariectomized mice there is a proportional increase in the activity of glucuronidase and the growth of the uterus at the several dose levels used.

Doses of α-œstradiol administered to ovariectomized rats in amounts sufficient to maintain the uterus at near normal weight were insufficient to maintain the glucuronidase activity above the castrate level.

Small doses of progesterone inhibit the stimulating effect of diethylstilbœstrol on weight and glucuronidase activity of the uterus ; large doses cause stimulation of uterine weight and glucuronidase, particularly when accompanied by minute quantities of œstrogen.

Progesterone inhibits the action of glucuronidase *in vitro*. This effect is not shown with the other steroids studied, i.e., [illegible] -one, 1-methyl-α-œstra- [illegible] to the possible physiological factors associated with elevated glucuronidase activity. It was suggested that glucuronidase may be involved in the accumulation of water in tissues through its possible role in the metabolism of intercellular ground substances.

REFERENCE

STAFFORD, R. O. (1949). *The Influence of Hormones on Enzymes in Some Endocrine Target Tissues* Thesis submitted for the degree of Doctor of Philosophy, Univ. Wisconsin.

DISCUSSION

FISHMAN: I'm very much interested in the water hypothesis because it may help explain some of the results that we've found in toxæmic women. To the gynæcologist the first symptom that presents itself in

nancies and abnormal pregnancies he seemed to find serum glucuronidase correlated with this gain in weight in several women. That observation made us look into the current theories of causation of toxæmia in pregnancy. As a rule the reports show that the urine of these women contains abnormally low amounts of pregnanediol and other steroids. The hypothesis which is widely accepted is that there is somehow a breakdown of the normal production of steroids. I think those data can be explained equally well on the basis that the steroids are formed in the normal amount but are not being excreted. They are being retained in the tissues of these women, with a resulting increase in water. And when we come to examine the literature as to what relationship there may be between œstrogens and water retention in the tissues, we find that certain people in this country, for example, have shown a very good relationship between œstrogen and water retention in tissues The sexual skin of the monkey becomes turgid during œstrus and it has been shown that there may be increased amounts of hyaluronic acid formed. All this has made us suspect a relationship between œstrogen, glucuronidase and mucin. So in the experiments that you described today it seems to me that there is direct evidence in support of these relationships. The fact that you find that 0 5 μg. of œstrogen is necessary for maintaining the blood level makes me think that perhaps in the early increase in uterine weight in the Astwood procedure, there is not sufficient œstrogen to produce an appreciable glucuronidase response. Certainly the amounts that we use under those assay conditions are well below 0·1 μg, about 0·05 μg.

FOLLEY: Did you try deoxycorticosterone glucoside in the *in vitro* experiments? That is a water-soluble derivative, and would do away

FOLLEY: Is that a detergent?

MEYER: Yes.

BUSH. I think that you get quite stable emulsions, at any rate with progesterone, using very minute quantities of alcohol. You can dissolve it in 80 per cent alcohol and then add a considerable volume of water so that the percentage of alcohol finally is only around 2 per cent, and then you get an emulsion which is stable and can be used for injection.

MEYER. We did some preliminary experiments on suspensions from a quantitative point of view. We didn't know how much we had in solution, so we settled on 10 per cent alcohol because these concentrations gave a water clear solution. Some investigators have just ground up the steroids and homogenized the tissue and then recorded the effects, on the assumption that the maximum was in solution.

WILLIAMS-ASHMAN: Have you tried ethylene glycol mono-ethyl ether as a solvent? It is supposed to be non-toxic.

MEYER: No.

FOLLEY: Has anyone tried to find a histochemical method for glucuronidase?

MEYER: That is what Friedenwald and Becker used.

FOLLEY: What is the method?

FISHMAN: They used 8-hydroxyquinoline glucuronide as the substrate and incubated slices of fresh tissue in the presence of this substrate plus an excess of ferrous iron. The iron reacts with the liberated hydroxyquinoline, precipitating in the tissue. Ultimately this iron is converted to Prussian blue.

Other people in Boston are developing histochemical methods for glucuronidase, and they have told me that glucuronidase stains well in epithelial, endometrial and glandular tissue, as well as in the epithelial malignant cell.

THE GROWTH INHIBITING ACTION OF CANCER PRODUCING SUBSTANCES IN RELATION TO HORMONAL CONTROL OF PROTEIN AND CARBOHYDRATE METABOLISM

L. A. ELSON

THE body growth and tumour growth inhibiting action of cancer producing substances such as 1:2:5:6-dibenzanthracene, 4-dimethylaminostilbene, etc., has been shown to be related to the protein content of the diet (Elson and Warren, 1947; Elson, 1948). In rats maintained on a high (20 per cent) protein diet, injection of the carcinogen usually has little immediate effect on body growth, although a delayed action resulting in rapid loss of weight, often followed by death, may occur later. Animals maintained on a low (10 per cent) protein diet, however, usually respond to the injection by an immediate, often prolonged, retardation of growth. The mechanism of the growth inhibiting action of these substances appears to be different from that of œstrogenic hormones like stilbœstrol, which was not found to be influenced by the protein content of the diet (Elson and Warren, 1947) and, unlike that of carcinogens, may be caused by antagonism of the anterior pituitary growth hormone (see Griffiths and Young, 1942).

It is suggested that the growth inhibition caused by cancer producing substances is a result of interference with protein metabolism, possibly directly with some enzyme process concerned in protein synthesis or through an action on nucleic acids which may act as intermediaries in the synthesis of special proteins, since Elson and Harris (1947) have shown an interference with the normal ratio of pentosenucleic- to deoxypentosenucleic acid in the livers of rats treated with 1:2:5 6-dibenzanthracene.

Another effect of this carcinogen on rat liver is to cause an increase in ascorbic acid content. This increase was found to be greatest in animals maintained on a 10 per cent protein diet, with which the maximum growth-inhibitory action of the compound was observed (Elson, Kennaway and Tipler, 1949).

Thus, as a result of this and other evidence concerning the toxicity of 1:2:5:6-dibenzanthracene in rats maintained on high and low protein diets (Elson, 1949), it appears possible that the carcinogens may interfere with the normal hormonal regulation of protein and carbohydrate metabolism, not, as is likely in the case of œstrogens, by antagonism of protein anabolic hormones themselves, but by a more direct interference with the action of some protein synthesizing enzymic systems under their control. It is thus possible that the carcinogens may be preventing the utilization of energy rich phosphate bonds for protein synthesis, and the energy rich phosphate is then available for an increased carbohydrate metabolism, of which there is some evidence in treated animals. Indeed, the increased liver ascorbic acid may well be related to this increased carbohydrate metabolism.

There is a considerable amount of evidence that carcinogens such as the polycyclic hydrocarbons and aminostilbene derivatives exert their biological action through a "toxic" metabolic product, and the action of these carcinogens can thus be considered as a dynamic process in which the rate of formation of the toxic metabolite depends to some extent on the rate of metabolism of the animal as a whole. The toxic metabolite itself upsets the balance of energy distribution between protein and carbohydrate metabolism which is normally regulated by hormonal influence.

The possibility that glucuronic acid and glucuronidase play an important part in these metabolic processes is suggested by the observations of Elson, Goulden and Warren (1946). In studying the excretion of aromatic amines it was found that the simpler amines such as aniline, 4-chloroaniline, etc., when administered to rats in small doses are excreted almost entirely as ethereal sulphates of the corresponding phenol derivatives.

These amines are not carcinogenic. Compounds containing more than one aromatic ring, however, such as 4-amino-diphenyl, benzidine and 4-aminostilbene, were found to be excreted as glucuronides and some of the free phenol derivatives were also found in the urine. Benzidine, 4-aminostilbene and the *N*-dimethyl-4-amino-diphenyl have been shown to be carcinogenic; aminostilbene and its derivatives in particular producing a large variety of tumours in many organs of the rat.

In this connection I would like to suggest that glucuronic acid formation and the role of glucuronidase in animal metabolism may not be exclusively concerned with the so called detoxification mechanisms, but may also be that of providing a transfer mechanism for conveyance of a fat-soluble but water-insoluble substance such as a steroid hormone from one organ in the body to others on which it is required to act. The substance is transferred by being converted into a water-soluble glucuronide, which may no longer show hormone activity, and this is carried in the blood stream, and, in this water-soluble form, is able to enter the cells of various organs. The active hormone is then liberated *in situ* in that organ by means of the glucuronidase present in the cell. On this conception the rather vague relation of glucuronidase to growth may be explained by the growth effects being really caused by the liberated hormone, and thus only indirectly related to the enzyme. Thus we would have, instead of the more usually accepted process of a hormone, regulating enzyme activity, in this case, the hormone actively regulated by the enzyme. This regulation could be controlled by the varying amounts of glucuronidase in the different tissues and/or by the presence or absence of an inhibitor of glucuronidase. The presence of such inhibitors in blood serum, etc., is probably significant in this respect. An [illegible] regulation of hormone

[illegible] isualized.

[illegible] simpler non-

[illegible] change from

the main form of excretion as ethereal sulphates to that of glucuronides occurs, may be highly significant, since transference as the water-soluble glucuronide and liberation of active carcinogen by the glucuronidase present in the various tissues could then occur. Excretion as glucuronide may thus be of marked importance for the production of tumours in such a number of diverse organs as occurs with this type of carcinogen.

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DISCUSSION

YOUNG: Was the food intake observed in all these experiments?
[illegible]

inhibition, but not all, because some animals maintain or even increase their food intake under treatment, when their growth is completely inhibited.

YOUNG: Did you analyse the total body content of protein or of fat?
ELSON: No. We didn't.
YOUNG The overall weight of the animal may not necessarily be significant with respect to protein metabolism.
FOLLEY: Are these carcinogenic compounds œstrogenic at all in large doses?
ELSON: No. There is a slight suggestion, but very slight.
FOLLEY: Do you know of any effect of these compounds upon pituitary cytology?
ELSON: No
YOUNG: You think that stilbœstrol suppresses the activity of the [illegible]

These amines are not carcinogenic. Compounds containing more than one aromatic ring, however, such as 4-amino-diphenyl, benzidine and 4-aminostilbene, were found to be excreted as glucuronides and some of the free phenol derivatives were also found in the urine. Benzidine, 4-aminostilbene and the *N*-dimethyl-4-amino-diphenyl have been shown to be carcinogenic; aminostilbene and its derivatives in particular producing a large variety of tumours in many organs of the rat.

In this connection I would like to suggest that glucuronic acid formation and the role of glucuronidase in animal metabolism may not be exclusively concerned with the so called detoxification mechanisms, but may also be that of providing a transfer mechanism for conveyance of a fat-soluble but water-insoluble substance such as a steroid hormone from one organ in the body to others on which it is required to act. The substance is transferred by being converted into a water-soluble glucuronide, which may no longer show hormone activity, and this is carried in the blood stream, and, in this water-soluble form, is able to enter the cells of various organs. The active hormone is then liberated *in situ* in that organ by means of the glucuronidase present in the cell. On this conception the rather vague relation of glucuronidase to growth may be explained by the growth effects being really caused by the liberated hormone, and thus only indirectly related to the enzyme. Thus we would have, instead of the more usually accepted process of a hormone, regulating enzyme activity, in this case, the hormone actively regulated by the enzyme. This regulation could be controlled by the varying amounts of glucuronidase in the different tissues and/or by the presence or absence of an inhibitor of glucuronidase. The presence of such inhibitors in blood serum, etc., is probably significant in this respect. An elaborate mechanism for fairly accurate regulation of hormone activity in different organs could therefore be visualized.

The observation that, in passing from the simpler non-carcinogenic amines to the carcinogenic ones, a change from

THE EFFECTS ON ENZYMES OF ADRENAL CORTEX, DIET, ŒSTROGENS, AND EXPERIMENTAL DIABETES*

CHARLES D. KOCHAKIAN

Dr. Kochakian presented data from many experiments by himself and co-workers. Most of this material has been published elsewhere, and summaries of these publications, prepared by Dr. Kochakian, are given below.

Adrenal Cortex

THE EFFECT OF ADRENALECTOMY, ADRENAL CORTICAL HORMONES, AND TESTOSTERONE PROPIONATE PLUS ADRENAL CORTICAL EXTRACT ON THE ARGINASE ACTIVITY OF THE LIVER AND KIDNEY OF THE RAT. *J. biol. Chem.*, 169, 1-6 (1947) Charles D. Kochakian and Virginia N. Vail.

ADRENALECTOMY decreased the fasting urinary nitrogen excretion and the arginase activity of the liver and kidney of young (150 g.) adult male rats. The administration of 1 per cent sodium chloride as drinking water was ineffective, and deoxycorticosterone acetate, 1 mg. per day, had a slight alleviating effect on the decrease in kidney arginase only. The administration of adrenal cortical extract (aqueous, Upjohn) at hourly intervals for 8 hours on the 5th postoperative day greatly increased the urinary nitrogen but did not affect the liver arginase, and partly restored the kidney arginase. Previous treatment with testosterone propionate, 2·5 mg. twice per day, did not alter the effects of the adrenal cortical extract but greatly increased the kidney arginase.

*The work reported in this paper was support[illegible]

pituitary extract could cause resumption of growth in animals where the growth had been depressed or prevented by administration of œstrogens.

KOCHAKIAN: I have often thought it was more an effect upon the food intake.

FOLLEY: But you do get cytological changes in the pituitary on œstrogen administration. One would think there would be a profound effect on the secretory mechanism

YOUNG: I am sure there are profound effects on the pituitary-secretory mechanism with respect to gonadotrophin, for instance, but I don't think as regards growth hormone that there is any clear evidence. We have a good deal of unpublished work which suggests that there may be some slight effect which is not merely the result of changes in the food intake, but the differences are so small as to be very doubtful

ELSON: What do you feel about the possibility of the inhibitory action being through an effect on bone growth?

YOUNG: Griffiths and Young found that growth of bone was depressed but not prevented. It is slow and may go on even when the body weight is falling.

A pellet of 11-deoxycorticosterone acetate produced a small increase in kidney weight and a small decrease in liver arginase, but 11-deoxy-17-hydroxycorticosterone was ineffective.

THE EFFECT OF ADRENALECTOMY, ADRENAL CORTICAL HORMONES, AND TESTOSTERONE PROPIONATE PLUS ADRENAL CORTICAL EXTRACT ON THE "ALKALINE" AND "ACID" PHOSPHATASES OF THE LIVER AND KIDNEY OF THE RAT. *Amer. J. Physiol.*, 150, 580-587 (1947) Virginia N. Vail and Charles D. Kochakian.

Adrenalectomy slightly increased the "alkaline" (pH 9·8) phosphatase of the liver of young (150 gram) adult male rats. The hourly injection for eight hours of adrenal cortical extract (aqueous Upjohn) on the fifth post-operative day greatly increased this enzyme. The increase occurred at a much faster rate than the increase in glycogen. Histochemical studies demonstrated greater amounts of the enzyme to be present (produced?) in the cytoplasm, walls and nuclei of the liver cells. Previous treatment with testosterone propionate did not alter the effect of the adrenal cortical extract on either the amount of glycogen or the increase in the enzyme activity. Deoxycorticosterone acetate was ineffective.

Adrenalectomy resulted on the fifth post-operative day in a small decrease in the "alkaline" phosphatase of the kidney which was prevented by the administration of 1 per cent sodium chloride as drinking water or by the daily injection of 1 mg. of deoxycorticosterone acetate. The hourly administration for 8 hours of adrenal cortical extract on the fifth post-operative day was ineffective Testosterone propionate, 2×2·5 mg./day, produced a marked increase in this enzyme.

None of the above treatments produced a significant change in the "acid" phosphatase (pH 5 4) of either the liver or the kidney.

EFFECT OF HIGH PROTEIN AND HIGH CARBOHYDRATE DIETS ON THE ARGINASE AND PHOSPATASES OF THE LIVER AND KIDNEY OF THE NORMAL AND ADRENALECTOMIZED RAT. *Amer. J. Physiol.*, 154, 489-494 (1948) Charles D Kochakian, Mary N. Bartlett and Jean Moe.

The feeding of either a high carbohydrate (89 per cent)—no protein, a high protein (casein 80 per cent, yeast 10 per cent)

THE EFFECT OF CRYSTALLINE ADRENAL CORTICAL STEROIDS, DL-THYROXINE, AND EPINEPHRINE ON THE ALKALINE AND ACID PHOSPHATASES AND ARGINASE OF THE LIVER AND KIDNEY OF THE NORMAL ADULT RAT. *J. biol Chem.*, 176, 243-247 (1948) Charles D. Kochakian and Mary N. Bartlett.

Aqueous (beef) adrenal cortical extract, lipoextract (hog adrenals), and 11-dehydrocorticosterone acetate produced very marked increases in the "alkaline" (*p*H 9·8) phosphatase of the liver of fasted rats when injected eight times at hourly intervals. The increase in enzyme activity did not parallel the degree of glyconeogenesis. Thyroxine produced a marked depletion of liver glycogen and a decrease in the enzyme. Epinephrine produced a tremendous deposition of liver glycogen but did not affect the activity of the enzyme.

In none of the above treatments were the activities of the arginase and "acid" (*p*H 5·4) phosphatase of the liver or the enzymes of the kidney altered.

CORTICOIDS AND BODY AND ORGAN WEIGHTS, NITROGEN BALANCE AND ENZYMES *J. biol. Chem.*, 190, 481, (1950) Charles D Kochakian and Evangeline Robertson.

The *stimulation of rapid glyconeogenesis in mice by* corticoids did not alter the arginase activities of the liver or kidneys. On the other hand, a subcutaneously implanted pellet of 11-dehydro-17-hydroxycorticosterone acetate produced significant increases in both tissues after two days. The same increases were obtained when the enzyme was determined with $CoCl_2$ as the enzyme activator or by pre-activation at 50°C with $MnCl_2$. The alkaline phosphatase of the liver was not increased until after 7 days. The body weight of the treated mice decreased very sharply, accompanied by an increased nitrogen excretion, a complete disappearance of the thymus, and a maximal decrease in the size of the spleen; but the food intake was increased about 20 per cent, so that after 7 days the extra protein catabolism was no longer evident and the loss in body weight abruptly ceased.

11-Dehydrocorticosterone implanted as a pellet produced effects qualitatively similar but quantitatively much less than those of 11-dehydro-17-hydroxycorticosterone acetate.

Holland by Dr. Gerritzen under very strict conditions, hourly doses of food and water. He has tried to reverse the rhythm with regard to water and chloride. If I remember rightly, by illumination at night and

all the time, just resting in bed.

ZUCKERMAN: It is a fair assumption that anyone who crosses the equator and proceeds far enough south, will change his diurnal rhythm in the same way as practically every animal species which is moved across the equator will reverse its breeding season. I put my question because some years ago, in work carried out with Dr. Bourne, we showed that the adrenal cortex and the thickness of its various layers

strikes me that practically all these cycles, diurnal cycle or oestrous cycle, may be associated with variations in the activity of all the tissues of the body.

BROWNE: How rapidly do animals reverse their breeding season when they cross the equator?

ZUCKERMAN: Some animals will reverse in their first season, others will take two or three. I don't think it would be longer than three.

PRUNTY: May I revert to the question of growth factors? Have you any data, Dr. Forsham, on the effect of your adrenal weight stimulating material in animals as far as lipoid discharge or any other change in the adrenals might be concerned? And secondly, with regard to your particular experiment, are you quite satisfied that you haven't had any potentiation by one series of injections of the next series? I've been

ACTH in this patient as judged by 17-ketosteroid and 17-ketogenic steroid excretion. The ACTH was administered over a period of weeks, and some was given intravenously in the middle, so it wasn't apparently just due to an inability to give an immediate response. We tried this patient with ACTH plus growth hormone, for five days, without any obvious effect at all. There may be a variety of reasons for that: the ACTH may not be active, although we have used two preparations, and the growth hormone may have been given in too small a dose, although there was sufficient to cause a significant rise in the fasting blood sugar level.

d adrenal

We had

increase adrenal weight. I wonder, Dr. Forsham, whether you have any evidence on the effect of your horse pituitary preparation on mitotic activity?

FORSHAM: None. However, Rinfret, finding this factor to be quite similar to your adrenal weight factor, did extensive animal studies on it

purified Armour growth hormone did not lead to the deposition of lipid in the way that this special adrenal growth factor did

The other question that Dr. Prunty raised is an important one: Do small amounts of ACTH, given day after day, increase the responsiveness of the adrenal to subsequent stimulation with larger doses? Our findings have been the same as those of Dr. Prunty, namely that, varying with individuals, varying with the type of ACTH used, you can actually enhance the responsiveness of the adrenal simply by giving small doses of regular ACTH day after day. The enhancement of response is far less than what we found with a special pituitary preparation: it will increase the response 3- to 4-fold, whereas small amounts of ACTH will hardly double it. So we feel that there is a possibility that one is dealing here with a special growth enhancing factor of the adrenal cortex.

SAMUELS. Dr. Eik-Nes was not able to find the same result with Rinfret's preparation in dogs. He found that he did get an increased blood corticosteroid response with a standard intravenous ACTH dose after the Rinfret preparation had been given for several days But when he gave the ACTH activity, which the Rinfret preparation assayed, over the same period of time to the dogs, he got the same enhancement of the blood response as after the earlier test.

FORSHAM. That again demonstrates the point that Dr. Prunty made. However, the preparation that you obtained from Rinfret was a mixture of conventional ACTH and the "adrenal growth factor", termed EAPE or equine anterior pituitary extract, prepared by the Victory Packing Company of Los Angeles, wasn't it?

SAMUELS. Yes

purified preparation which had very little steroidogenic effect, and

enhanced adrenal responsiveness much more than would correspond to its steroidogenic equivalent of ACTH.

SAMUELS: With ACTH given intramuscularly in gelatin for a period of seven to ten days we certainly got an enhancement in response to intravenous ACTH.

BAYLISS: Dr. Forsham, in your abstract you mentioned "suitable

we couldn't actually prove it until we did this quantitative study plotting the dose-response curve. You may recall that there were six perfectly normal medical students used, four of whom had response

BAYLISS: If you repeat the test on those same six medical students, do you get the same pattern of response?

FORSHAM: Yes. We had to do that for our assay. After eight months

a group of four. So apparently in a controlled group one gets better reproducibility than we expected.

THORN: Did you ever check this same group with intravenous assay, to see if this adrenal unresponsiveness was purely an intramuscular effect?

FORSHAM: No, we didn't. It is a most important point to be settled.

ZUCKERMAN: Did I understand you to say that pitressin masks the action of ACTH?

FORSHAM: No, it prolongs the excretion of urinary corticoids. With a very pure preparation of ACTH there was a rapid fall in urinary corticoids as soon as the eight-hour intravenous administration was stopped. When this same preparation was given with one unit of pitressin the elevated urinary corticoid excretion persisted significantly longer. This may well be a renal effect in part, whereby antidiuresis retards steroid excretion.

nicely in the way we ran our experiments. I should like to present a slide (Table I) to show the quantitative relationship between the sodium/potassium ratio and the ACTH administered. The experiment

to the dosage. The Na/K ratio might be a useful way of showing a response.

Table I

(Elmadjian)

RESPONSE OF URINARY Na/K TO DOSAGE OF ACTH*

Subject	I U, ACTH			
	5 0	25	50	150
Cn	2 7	1 8	1 3	0 8
SR . . .	4 0	1·7	0 7	1 0
SC	2 1	1·3	0·7	0 5
Cm	1 7	1 0	0·7	0·7
Mean	2 6	1 5	0 9	0·7

*Organon Corticotrophin Zinc Phosphate Twelve-hour collection ACTH given at 8 0 a m

e maximum

ELMADJIAN: No. They were students, and were not on bed rest. The

There was a twenty-four-hour control period and twenty-four-hour periods of ACTH injection We did eosinophil counts, the 17-ketosteroid increase, and the urinary sodium and potassium. The difficulty was, of course, that these boys were on a normal food intake. They were asked to keep their food intake as constant as they could over a period of one week. We did a cross-over experiment with the two ACTH preparations so that each subject received both samples of ACTH We came to the conclusion that the 17-ketosteroid excretion was a poor index in this experiment. The time of observation was probably too short. The only one that really was of any value was the eosinophil fall, and that correlated extremely well with the Sayers test. I think the sodium/potassium ratio was probably vitiated to some extent by the dietary conditions mentioned.

QUERIDO: I am astonished to hear these complaints about 17-ketosteroid excretion. We have done extensive studies on the 17-ketosteroid excretion under controlled conditions. If you give the patient a three-day control period, and then a three-day injection period, and then a three-day control period, or do it in a series, with several other preparations between two standard preparations, we have come to the conclusion that actually the 17-ketosteroid excretion is a good measure, not for glucocorticoids I must say, but for adrenal stimulation. Even [illegible] for glucocorticoid production than the eosinoph [illegible] 17-ketosteroids are stil [illegible] the eosinophils. Bu [illegible]

FORSHAM: I think [illegible] tion with, 17-ketosteroids only on the basis [illegible] long time intervals for collection. That is the crucial point. If one wanted to follow quick changes in adrenocortical activity, such as the hourly dynamics, then I think one does much better with the 17-hydroxycorticoids.

VARIABILITY OF ADRENOCORTICAL RESPONSE TO ACTH IN DIFFERENT PERSONS, AND THE INFLUENCE OF VARIATIONS IN ADMINISTRATION OF ACTH PREPARATIONS ON THE LEVEL OF 17-HYDROXYCORTICOSTEROIDS IN THE BLOOD

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BEFORE drawing conclusions about the influence of ACTH preparations on adrenal activity, it is imperative to realize what the indices that are used mean, and how variable they are in the same person or from person to person. In this paper we wish to limit our discussion to the problem of assessing so-called glucocorticoid production.

There are in general three types of measurement of hormone production: the level of the product determined in the blood; the excretion of related substances in the urine during a certain period; and quantitative assessment of the metabolic effect exerted by the hormone that is being studied. Each of these methods has its limitations. The estimated level in the blood is the result of both production and rate of disappearance from the blood through excretion and metabolism. Studies of the urinary metabolites have the advantage that the production is reflected in the time element of specimen collection. However, the disadvantage of urinary determinations is in general that the metabolites measured are only a reflection of the compound under investigation. The third method, quantitative assessment of the metabolic effects, introduces still more variables.

In the past we have mainly collected data on evaluation of adrenal stimulation after ACTH treatment, with the aid

of urinary neutral 17-ketosteroid (17-k.s.) excretion. It is a well known fact that urinary twenty-four hour 17-k s. excretion is rather constant from day to day in the same person.

Summarizing our results (which have recently been published, Goslings, Querido, Cats and Kassenaar, 1954) we want to show the variability of the 17-k.s. response seen with the same batch of ACTH in different subjects (Table I). Expressed in rough percentages it appears that variations from 100–600 per cent increase in 17-k.s. may be seen with the same dose of ACTH. This makes it impossible, for example, to compare different ACTH preparations in different persons. We are forced to carry out such assays in the same individual. The scheme adopted for these assays is shown in Fig. 1. We consider it necessary to repeat the first series of injections in order to minimize the possibility of variations in the subject during the time that the experiment is being done. The result of such an assay is seen in Fig. 2.

Table I

VARIATION IN 17-KETOSTEROID EXCRETION IN DIFFERENT PATIENTS TREATED WITH THE SAME BATCH OF ACTH (6×7 I U. CH. 22/G).

Patient	*17-Ketosteroids*		
	Before ACTH (mg /24 hr)	*After ACTH (mg /24 hr)*	*Increase*
Sc.	10 2	20·0	100
St	10 0	21 1	110
Gu	7 0	18 5	120
C.	8 7	24 0	180
Ge.	8 0	24 1	200
Kn.	4 2	12·7	200
V	11 8	35·3	200
Ra.	13 8	55 9	310
P.	7·9	34·1	330
v. R.	6 0	31 8	430
Mi.	4 7	26 5	450
L.	4 8	28 2	500
Sw.	4 2	30 5	620

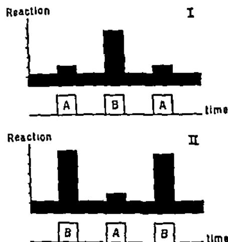


FIG. 1. Scheme by which different preparations of ACTH are compared.

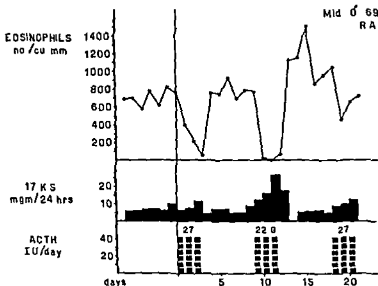
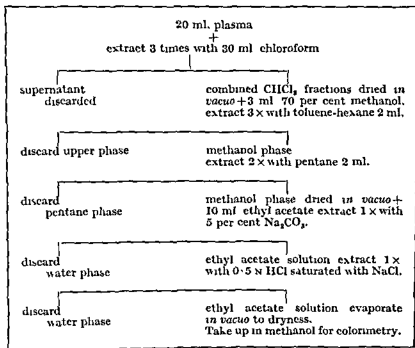


FIG. 2. Comparison of different batches of corticotrophin injected in the same patient at different times.

Since we actually wanted to know glucocorticoid production after ACTH injections, we recently tried to compare 17-k.s. excretion after ACTH administration with the 17-OH corticoids (17-OH CS) values in the blood. Dr. Kassenaar and co-workers (Kassenaar, Moolenaar and Nijland, 1954) developed in our department a method based on the Porter-Silber reaction, after purification of the blood extract by liquid/liquid partition (Table II). The correction procedure

Table II

FLOW SHEET FOR THE PURIFICATION OF PLASMA EXTRACTS PRIOR TO PORTER AND SILBER REACTION (KASSENAAR C. S.).



of the colorimetry is done rather extensively through measuring the absorption curve of the coloured compound between 3700 and 4600 Å, taking readings at 100Å intervals. The method has the advantage that only pure chemicals are used and that it is unnecessary to rely on commercial adsorbents.

The recovery is quite satisfactory. Duplicates never differ more than 1 μg. per 100 ml. The values for normal persons varied from 4·7–10·2 μg. in females and from 5·0–9·5 μg. in males (see Table III). The blood was collected in the first part of the morning.

Table III

17-OH CORTICOID VALUES OF PERIPHERAL BLOOD IN μG./100 ML. PLASMA OF NORMAL INDIVIDUALS

	Males	*Females*
No. of observations	21	16
Mean ±s.d.	6 8±1·4	6·2±1 5
Range	5 0—9 5	4 7—10·2

Recovery experiments

No of experiments	*Amount added*	*Mean recovery*	*Range*
14	5 μg. Cmpd. E	4 65 μg =93%	80—104%

The data in diseases of the adrenal cortex were very promising. In patients with Cushing's disease our values were elevated, while three patients with classical Addison's disease all showed low figures In three cases of pseudohermaphroditism we also found a low level in the blood (Table IV).

To measure intra-individual variations of this index we studied the 17-OH CS levels in the plasma at different hours of the day in the same persons under constant conditions. Normal subjects were fed every hour 30 ml. of water and 10 grams of cheese, 5 grams sugar and a small biscuit (total 94 calories). During this experiment they stayed in bed, and had to empty their bladder each hour. This technique, mainly developed by Gerritzen (1939), produces a rhythmic excretion of water, sodium and potassium (Fig. 3). There also is a very clear diurnal variation in neutral 17-k.s. excretion (as previously shown by others) both in quantity and in concentration. It now appears that the same diurnal variations can be seen

Table IV

17-OH CORTICOIDS IN PLASMA (μG /100 ML.) IN ADRENOCORTICAL DISEASES

Patient	*Sex*	*Diagnosis*	*Result*
Osna	♀	Cushing's Disease	17 1
,,	♀	,,	16·5
,,	♀	,,	8 3 (after removal of tumour containing 4 5 μg./g. tissue).
Hoefl.	♀	,,	10 0
Ha	♀	,,	14 0
To	♀	,,	13 0
Meu		Adrenogenital Syndrome	2 0
Mey		,,	nil
Bre		,,	2 4
Am	♂	Addison's Disease	1 6
Ba	♀	,,	1 7
Ka.	♀	,,	nil

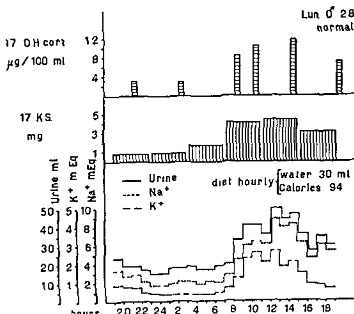


FIG. 3. 24-Hour rhythm of urine production, sodium, potassium and 17-k s. excretion and plasma 17-OH CS in a normal man.

in the 17-OH CS level of the blood. We have been able to observe this phenomenon in the five normal young men who have been subjected to this study. In three cases the peak was reached at 2 p.m. and in two at 8 a.m. In all cases, however, the peak of water and mineral excretion was at noon. This diurnal variation in 17-OH CS in the blood has been discussed by Bliss and co-workers (Bliss, Sandberg, Nelson, Sweat, and Eik-Nes, 1953). It is difficult for us to evaluate their data since a different method has been used by our laboratory. For example, they found intra-individual variation from day to day on the same hour to be greater than we did. The arrangement of the experimental conditions is different, and this might explain their findings of the highest values nearly always at 8 a.m.

An interpretation of this phenomenon is difficult. Referring to our introductory remarks on the meaning of blood levels, we want to emphasize again that this result does not necessarily indicate diurnal variation of the production of 17-OH CS by the adrenal cortex. In the normal person subjected to the above mentioned test, creatinine excretion does not show diurnal variation, which may be considered an indication that glomerular filtration at least does not show rhythmic variation. Blood sodium and potassium levels are constant throughout the day, and therefore it seems probable that the rhythmic variation in the mineral excretion is dependent on variation of tubular function. Sandberg, Nelson, Glenn, Tyler and Samuels (1953) showed, when collecting eight-hour specimens, that the highest excretion was found in the 7 a.m. to 3 p.m. sample. This coincides with the interval of the highest blood 17-OH CS values. We are inclined to accept this observation as possible evidence that the 17-OH CS peak in the blood is not induced through reduced renal excretion. The question remains to be answered whether the diurnal changes in 17-OH CS are due to changes in production or metabolism.

The aim of the study was to compare 17-OH CS values in the blood with the excretion of neutral 17-k.s. under

conditions of ACTH administration. A female patient (Os.), forty years of age (Fig. 4) was given 6×4 i.u. ACTH (Res P 277) intramuscularly during three days. On the morning of the fourth day one more injection of ACTH was given at 8 a.m. and blood samples were taken at 9 and 11 a.m. There was hardly any rise in 17-k.s. excretion but a very clear rise in 17-OH CS in the blood. Another preparation (Ch. 37), given at higher dosage, produced a still further rise in 17-OH

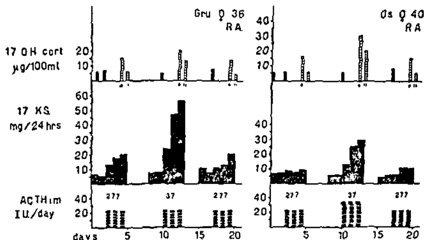


FIG. 4. Comparison of different preparations of corticotrophin on the 17-k.s. excretion and 17-OH CS level in blood in two patients.

CS and an increased 17-k.s. excretion. The percentage increase of the 17-OH CS was probably greater than the increase of the 17-k.s. From this experiment it was evident that the control values for 17-OH CS from day to day in the same person did not vary appreciably (black columns). The effect of one injection of ACTH intramuscularly seemed to fade away after three hours. Another patient (Gru., female, forty years of age) on the same scheme of treatment, also studied with intramuscular ACTH injections of the same batches (Res P 277 and Ch. 37) showed a quite different pattern. In this case the effect of the 17-k.s. excretion was more pronounced than on the 17-OH CS in the blood.

Similar discrepancies between blood 17-OH CS response and 17-k.s. excretion have been observed with intravenous infusion of ACTH in 5 per cent glucose 50 ml./hour during twenty-four hours (Fig. 5). One-third I.U. ACTH per hour (male patient, twenty-two years of age) had no effect on the 17-k.s. excretion and a very appreciable effect on the 17-OH CS value in the blood. Increasing the dose from 1/3 I.U. per hour to 1 I.U. per hour hardly changed the 17-OH CS level,

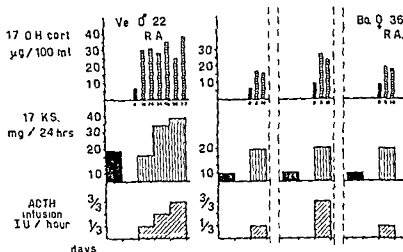


FIG. 5. Excretion of 17-k.s. in urine and blood level of 17-OH CS in two patients after a corticotrophin infusion.

but it did increase the 17-k.s. excretion. In another person (female, thirty-six years of age) there was an increasing effect on the 17-OH CS blood level with increasing dose of administered ACTH, while the 17-k.s. stayed constant.

If we assume that increase of blood 17-OH CS level is always reflected in increase of 17-OH CS excretion in the urine, our data are not easily reconciled with those of Sandberg and co-workers (Sandberg, *et al.*, 1953). After ACTH these authors always found greater response of urinary 17-OH CS excretion than of 17-k.s. excretion.

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DISCUSSION

DELTOUR: Concerning the variability of the adrenocortical response, have you noticed any sign of acute adrenal insufficiency on perfusing with ACTH a patient with Addison's disease? We have seen two accidents with 20 units of a commercial preparation of ACTH. The first

QUERIDO. No, we do not give ACTH infusions to Addisonian patients in our Department

THORN: Any intravenous infusion in an untreated patient with Addison's disease is a calculated risk, and ACTH given intravenously can make an Addisonian patient very ill. I have seen both types of reaction that Dr. Deltour described: the allergic reaction to intravenous

should only be carried out under very rigid, carefully prepared circumstances where the evaluation justifies the administration of the material.

FORSHAM: I might add one tragic instance of the danger of present-day corticotrophin gel in patients with adrenal insufficiency. There is a fair amount
called highly
woman suspe
equivalent units
Unbeknownst to us the patient began to lose sodium at a rather tremendous rate while retaining water. After nine days she suddenly

throughout that period

Querido: I agree fully.

Wilkins: I should like to ask Dr. Forsham whether in repeatedly

parations If we used less pure preparations, this work would be impossible. We did find developing sensitivity, and one might also stress the subject's own pituitary into action.

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fir

many years before she had had two or three injections of a crude anterior pituitary extract Since that time, in patients with rheumatoid arthritis, we have had two or three reactions to intravenous ACTH of a fairly severe nature, anaphylactoid type, drops in blood pressure and generalized urticaria, that were very frightening.

Prunty: Could I return to the 17-ketosteroids? Professor Querido gave the percentage increases in different individuals treated with ACTH (Table I). There are two factors in these increases which I think one must take into consideration: the initial level and how high it goes. A percentage increase is not a valid representation of change in adrenal function A better approximation we think is to take the mg. rise, if you want to compare one person with another, but even so you still have to think about base levels.

Elmadjian May I bring out the point again that the 17-ketosteroids are a mixture of compounds. The type of experiment which I think would help us considerably would be to do the blood Porter-Silbers, the urine Porter-Silbers, the 17-ketosteroids and chromatograph them, to see the conversion of Porter-Silber chromogens to 17-ketosteroid titres

such as the Δ^{9}-aetiocholanolone type of compound, and furthermore to see what the *ratio* of the 17-ketosteroids to the Porter-Silbers in the urine is during these three-day procedures. I think Professor Querido has some interesting data which will help us because of the time interval he has chosen. Three days of injection may assist a great deal in elucidating this conversion problem.

FORSHAM: I'd like to rise to the defence of Professor Querido. One gets the impression that we are all talking down the 17-ketosteroids. I don't think it really makes any difference what you measure over a matter of three days, as long as there is some relation between the dosage used and your parameter. I might point out that all of us have relied for years on ACTH potencies expressed on an assay based on the discharge of ascorbic acid from the rat adrenal, which has absolutely nothing whatsoever to do with steroidogenesis. Yet it was very valuable. I think one has to define what sort of an assay one wants. In terms of a

to the mechanism of the conversion of the 17-hydroxycorticoids to 17-ketosteroids, that is fundamental research, in which the data Professor Querido has supplied are going to help us a lot.

THORN: We have carried out a large number of assays now, and studied the normal response to ACTH, and if one uses either an eight-hour infusion on two successive days, or the gel, the rise in 17-ketosteroids and the rise in total 17-hydroxysteroids follow each other very nicely. However, one is likely to obtain a greater response in the 17-hydroxy on day 1.

BROWNE: In connection with blood levels in general, and Dr. Forsham's remarks about pitressin action, and the drop in the hæmato-

concentration would be affected in the same way as any other blood constituent

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as great as one might expect,

logarithmic. In regard to the distribution of hydrocortisone in the

between cortisol (or cortisone) and the plasma proteins.

one and three-quarter hours and in the arterial about one and a quarter to one and a half hours. It seems to take about three hours for a molecule to go through the metabolic circuit and out.

We find with the radioisotope that in the hepatic venous blood, which has a decay curve for free steroid about the same as peripheral venous blood (indicating about the same time through the portal system), we get a very nice rise in the conjugated material, which reaches a peak at about two hours and is still high at about four hours; and we find

normal subject without this outward drainage So that the intestinal factor is probably involved in the relation between the blood and urinary excretions. You have your peaks in the blood level at the same time as the peaks in the urine, and I am wondering whether that might have been a matter of time interval that I didn't follow.

QUERIDO: The urine was collected hourly, but we of course only studied water, sodium and potassium. However, I stated that in three cases the peak of the blood corticosteroid level was at 2 p.m. and in two

SAMUELS. I am very curious about the high levels around 2 o'clock in the afternoon. In our experience the peak levels in the plasma have always come between 6 and 8 a m , and we have never seen one beyond

that time. I wonder what other people's experience has been with diurnal . nething ie same

FORSHAM: I suggest that we schedule a special meeting at Greenwich! This is really not so funny, because when we tried to compare our results in California with published results from Utah and elsewhere, we finally concluded that all local times would have to be changed to a common denominator to allow for proper comparison.

THORN: There appears to be considerable interest in the diurnal fluctuation, since many data have been accumulated on this subject. I would suggest that we reserve time later in the meeting for more extended discussion of this problem.

SAMUELS: I should like to make one remark with regard to 17- . which Professor Querido . we have been measuring d and the other is after glucuronidase hydrolysis. When we measure the 17-hydroxycorticoids we take off from our chromatogram the fraction that has 17-ketosteroids; this does not have the total 17-ketosteroids, only the glucuronides, and it is always considerably below the total level. Now in this study Dr. Sandberg was interested in the relation of urinary 17-hydroxycorticosteroids to the plasma levels, and I think the 17-ketosteroids were also done on the glucuronide fractions, which would make them considerably lower too.

QUERIDO: Would Dr. Samuels infer from his experiences on Compound F administration and the variations in the blood in different persons, that the blood levels are more or less a reflection of production?

SAMUELS. I think in the normal individual, yes. But in patients, no, because the liver function can greatly influence the levels.

BAYLISS: I think some of the disparity you have observed between the blood levels of 17-hydroxycorticosteroids and the 17-ketosteroid excretion is related to the rapidity with which the concentration of 17-hydroxy compounds may alter in the plasma. Relatively large volumes of blood are required for the estimation, and you can't take very many samples. Sometimes when you found high 17-ketosteroid excretion and the plasma level of corticosteroids was not apparently proportionately high, the discrepancy could be related to the timing of the blood sample. Would Dr. Samuels agree with that?

SAMUELS: Yes.

BAYLISS: That's why I think it is often difficult, unless you can take

four hours.

QUERIDO: I agree with you only partly, that this is a possibility. In the case I showed of the increased intravenous infusion—one day was

and six hours of infusion. Of course, you had two samples. If you plotted them logarithmically you'd probably find the same.

STUDIES OF THE INTER-RELATIONSHIP BETWEEN THE ADRENAL CORTEX AND ASCORBIC ACID METABOLISM

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Barbara E. Clayton, M.D., Ph.D.,
R. R. McSwiney, M.B., B.S.,
and
Ivor H. Mills, M.B., B.Chir., M.R.C.P., Ph.D.,

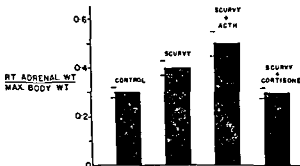
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The observations of Sayers and of Long (Long, 1947) on the decrease of adrenal cortical ascorbic acid in response to ACTH have been the basis of the acceptance of the existence of a specific relation between the hormone and this response. This has pointedly raised the question of the rôle of the ascorbic acid in the adrenal cortex and the method of disposal when ACTH is given.

Effect of Scurvy on Adrenal Cortical Function

The mere fact that conditions in the terminal scorbutic period in the experimental guinea-pig are sufficient to act as an intense stimulus of adrenal cortical activity indicates the possibility of adrenal cortical secretion in the absence of ascorbic acid. It was found that in the terminal stages of acute scurvy in guinea-pigs the 17-ketosteroid excretion rose (Clayton and Prunty, 1951). This is true in the monkey (Stewart, Salmon and May, 1952), and Nadel and Schneider (1952) found an increase in formaldehydogenic substances in the urine of scorbutic guinea-pigs. The excretion of 17-ketosteroids, *however, falls during the earlier scorbutic* period in both acute and chronically scorbutic animals. In scorbutic patients too, 17-ketosteroid excretion is depressed

and it has been suggested that a rise in 17-ketosteroid excretion might prove to be indicative of impending termination unless ascorbic acid therapy is given (Clayton, Mills and Prunty, 1954). This preliminary fall with an increase occurring only in the terminal phase may explain why other workers have failed to notice the rise (Banerjee and Deb, 1952; Tarantino, 1950). In the guinea-pig, depletion of adrenal ascorbic acid seems to occur at a rapid rate on a scorbutic diet. At the twelfth day the level had fallen from 81·7 ±



32·6 mg./100 g. to 5·4±6·2 and 0·94±2·4 in the "terminal period" of scurvy. For the purposes of the present study "terminal period" may be defined as the limits within which animals in our colony succumb with acute scurvy. In some animals, therefore, it is likely that adrenal ascorbic acid had reached zero levels at the twelfth day or very soon after. Oesterling and Long (1951), however, reported rather higher figures—after seventeen days the level was 6·02±2 and after twenty-seven days 1·87±0·5. Methods for determining ascorbic acid at these very low levels become extremely inaccurate. During the terminal period there is a significant increase in the size of the adrenal gland (Fig.1). This is

accompanied by the usually accepted evidences of "stress" response. There is discharge of sudanophilic material from the zona fasciculata. There occurs too a fall in the adrenal cholesterol level (Oesterling and Long, 1951) which may be detected as early as the fourteenth day (Stepto, Pirani, Consolazio and Bell, 1951). By paper chromatography of ethyl acetate extracts of guinea-pig whole blood a very large rise in the hydrocortisone level may be demonstrated which exceeds the amount secreted in response to the injection of ACTH gel daily into normal guinea-pigs. Much of the secreted

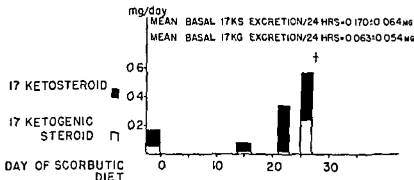


FIG. 2. 17-ketosteroid and 17-ketogenic steroid excretion in acute scurvy in the guinea pig.

hydrocortisone appears as unchanged free steroid in the urine (Burstein and Dorfman, 1954; Mills, 1954) and is able to undergo oxidation to 17-ketosteroid by sodium bismuthate and be estimated according to the method of Norymberski, Stubbs and West (1953). Using a modification of this method it is found that there is a decrease in 17-ketogenic steroid in the urine of guinea-pigs on a scorbutic diet about the twelfth day and a rise above normal levels at the end of the terminal period (Fig. 2). There seems little doubt, therefore, that some process associated with the terminal stages of scurvy is an intense stimulus to adrenal cortical activity which appears grossly to be of the normal pattern. This is in spite of the fact that the adrenal cortical content of ascorbic

acid is virtually zero. Unfortunately methods are not sufficiently sensitive to be able to state that adrenal steroid production is entirely independent of traces of ascorbic acid. There certainly seems to be no essential need for the very large amount normally present in the cortex. If ascorbic acid were important in steroid synthesis it might be expected to enhance hydroxylase activity. This effect appears to be minimal with respect to 11-hydroxylase activity according

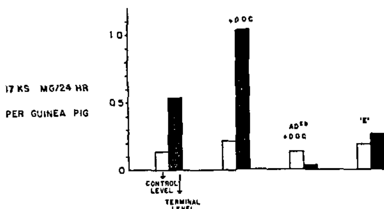


Fig 3. Effect of deoxycorticosterone acetate (D.O.C.), adrenalectomy plus deoxycorticosterone acetate (2 mg daily injected), and cortisone acetate ('E') (5 mg. daily injected) on the 17-ketosteroid excretion in scurvy in guinea pigs.

to the report of Wettstein, Kahnt and Neher at the first part of this colloquium.

It has been found that cortisone in suitable dosage will depress the hypertrophy of the adrenal developing in scurvy (Fig. 1) and the excretion of ketosteroid in the urine (Fig. 3). According to Sayers' hypothesis this would be due to the inhibitory effect of the cortisone on the pituitary secretion of ACTH. The amount of steroid required for this purpose increases with the increasing stimulus to endogenous ACTH production (Sayers, 1950). It will be seen in Fig. 3 that deoxycorticosterone acetate caused an increase in the 17-ketosteroid excretion in scorbutic guinea-pigs although in

normal guinea-pigs it does not have this effect. According to Sayers, deoxycorticosterone in half of the dose per 100 g. body weight employed in these experiments, given to the rat suppressed endogenous ACTH. Deoxycorticosterone was said to be one-eighth as effective as cortisone. In the present experiments DCA seems to act as an additional stimulus to ACTH production and this view is supported by observing the following changes in the weight of the right adrenal gland in scorbutic guinea-pigs:—

Table I

FEMALE TERMINALLY SCORBUTIC GUINEA-PIGS

R. adrenal gland. Ratio of weight in mg. to highest body weight in g.

Intact	*Intact + DCA**
0·38	0 60
0 41	0 50

*2 mg per day i m.

Adrenal Cortical Reserve in Scurvy

The injection of ACTH into guinea-pigs completely deprived of ascorbic acid during the nineteenth to twenty-third days of the regime caused a 40 to 206 per cent increase in the 17-ketosteroid excretion of these animals (Clayton and Prunty, 1953). ACTH treatment of animals on a scorbutic diet results in a still further increase in adrenal weight (Fig. 1).

In patients suffering from chronic scurvy there is a very rapid and impressive response to ACTH in the excretion of 17-ketosteroids and 17-ketogenic steroids (Fig. 4) equal to the best responses seen in normal individuals (Clayton, Mills and Prunty, 1954). It should be especially noted that in this response, which has been confirmed in two patients, the ketosteroids rose from low levels. It has also been reported by Treager, Gabuzda, Zamchek and Davidson (1950) that scurvy patients show a good eosinophil fall when given ACTH and that Gardner at the Peter Bent Brigham Hospital has observed a good rise in 17-ketosteroid during forty-eight

hours' administration of ACTH. We can be more certain of a very severe depletion of adrenal cortical ascorbic acid in the guinea-pig experiments, and, in spite of this, adrenal cortical reserve would seem to be good. The observations in patients may be accepted as supporting this conclusion.

The significance of the fall in urine 17-ketosteroid during the more chronic phases of scurvy is of some interest. One of

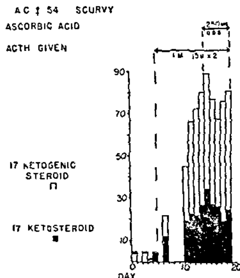


FIG. 4. Urine steroid excretion in a clinically scorbutic patient treated with ACTHAR gel.

our patients received 2 g. of ascorbic acid daily without any immediate increase in the urine 17-ketosteroid excretion; the average daily excretion was 2·8 mg. per day and during the first seven days' treatment was 3·0 mg. per day. Klein, Papadatos and Fortunato (1954) observed no consistent change in the normal human in 17-ketosteroid excretion when large doses of ascorbic acid were given. Kayahan (1952) on the other hand reported a fall. In our patient the 17-ketogenic steroids, which were also low, showed a slight

depression, but it is doubtful if this was significant, although others have claimed to observe changes of a similar nature with ascorbic acid (Daughaday, Jaffe and Williams, 1948; Goldzieher and Edlin, 1953).

Effect of Adrenal Hormones on Scurvy

The effect of the adrenal on the scorbutic state itself now deserves consideration and appears to be somewhat controversial. Early observations by Lockwood and Hartmann (1933), by Kendall (see Murray, 1948) and Giroud and his *colleagues (Ratsimanaga, 1944), seemed to show that adrenal* cortical extracts prolonged survival of guinea-pigs deprived of vitamin C, although the extracts may contain minimal amounts of this substance. In acutely scorbutic animals, Hyman, Ragan and Turner (1950) and Herrick, Mead, Egerton and Hughes (1952) have found that ACTH and cortisone prolong survival. Schaffenburg, Masson and Corcoran (1950) also, using only partially deficient animals, claimed that cortisone enabled growth to be maintained. The question whether scurvy is improved bý cortisone and ACTH has been studied by us, paying special attention to both acutely scorbutic animals completely deprived of vitamin C, and to more chronically scorbutic animals receiving 1 mg. of ascorbic acid every fourth day and other animals receiving 2 mg. of ascorbic acid daily. In acutely scorbutic animals 10–20 units of ACTH daily, in divided doses, had no significant effect on survival (Clayton and Prunty, 1951). Cortisone acetate in doses of 5 mg. daily subcutaneously had no effect on survival (Fig. 5). It is interesting to note, however, that, although as we have seen, DCA appears to potentiate the adrenal stimulus of acute scurvy, in doses of 2 mg. daily, it does not affect the survival of these acutely scorbutic animals. Adrenalectomized animals, which may be maintained on this dose of DCA (Clayton and Prunty, 1953), succumb extremely rapidly—even before a gross depletion of ascorbic acid in the tissues is likely to occur. This extreme susceptibility may be surprising, but it has been previously

pointed out that adrenalectomized animals maintained in this way are extremely likely to succumb to such unfavourable conditions, being sensitive to "stress" (Swingle and Remington, 1944). On the other hand scurvy itself sensitises the animal to such stimuli as *infections and the injection of* histamine (Sayers, 1950; Parrot and Richet, 1945) and it has been convincingly shown by Dugal and Therien (1949) that

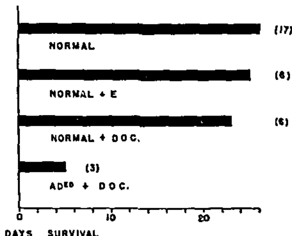


FIG 5. Survival of guinea pigs on a scorbutic diet. Dosages used were deoxycorticosterone acetate 2 mg. daily, cortisone acetate 5 mg. daily.

in rats and guinea-pigs large amounts of ascorbic acid prevent the adrenal hypertrophy produced by exposure to cold and favour the survival of such animals.

In the case of chronically scorbutic animals, the administration of 1 mg. of ascorbic acid every fourth day resulted in death between twenty-six and forty-five days and the administration of cortisone acetate in doses of 5 mg. daily did not alter the survival period outside this range. *Animals* receiving 5 mg. cortisone acetate and 2 mg. of ascorbic acid daily survived not more than fifty-two days, whereas animals not receiving cortisone were still alive at ninety days. It is

considered that more acute scurvy was not precipitated in the cortisone animals; indeed bone development at the epiphyses appeared stimulated (Clayton, 1954). In these experiments it was shown that the observations of Schaffenburg, *et al.* (1950) claiming that cortisone enabled growth to be maintained in chronically scorbutic animals, were dependent on free food intake. If paired feeding is employed, the cortisone animals even show an absence of the initial weight gain seen in the scorbutic controls. The falling off in food intake by these animals is very considerable. We have there-
fore, been
the surviv
pigs, altho
to the effects of the scorbutic diet. Reports in the literature on the effects of ACTH on scurvy are contradictory and unconvincing. In two scorbutic patients we treated with ACTH for four and nine days respectively there were no objective changes of note.

Effects of ACTH and Cortisone on Ascorbic Acid Metabolism

In both the human subject and the guinea-pig on good intakes of ascorbic acid the excretion of ascorbic acid in the urine is increased by ACTH injection (McSwiney, Clayton and Prunty, 1954). Fig. 6 shows the response in guinea-pigs receiving 10 mg. ascorbic acid per 100 g. body weight and Fig. 7 the response in a patient receiving 310 mg. of ascorbic acid daily. The response in the guinea-pig is more sustained than in the human. In the latter it is reminiscent of the increase in uric acid excretion often observed and of transitory nature. In Fig. 7 a prominent rebound is seen after withdrawing ACTH. Beck, Browne and MacKenzie (1951) observed similar changes in humans. We have amplified our observations by determining ascorbic acid with 2:6-dichlorophenolindophenol, and ascorbic acid plus the oxidation products of dehydroascorbic acid and diketogulonic acid, if present, by the method of Roe and Kuether (1943). It will

be seen that ACTH induces some increase in the amount of oxidation products (Roe and Kuether value—dye value). The contribution of dehydroascorbic acid to this was practically insignificant (McSwiney, Clayton and Prunty 1954). It was further found that a similar phenomenon occurs if the intake of ascorbic acid, and hence the urine excretion, is suddenly increased in a control subject, and it is therefore

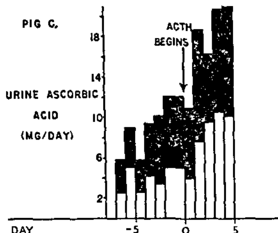


FIG. 6. Effect of ACTH on urine excretion of ascorbic

suggested that the fundamental change that occurs when ACTH is injected is an increase of ascorbic acid mobilised from the tissues. In Fig. 7 it will be seen that in two days the ascorbic acid plus oxidation products exceeded the intake by 300 mg. and the preceding baseline excretion rate by 454 mg., amounts far in excess of the adrenal content of ascorbic acid, which is approximately 12 mg. It would not be expected that such changes would occur in guinea-pigs for these animals excrete such a small proportion of ingested ascorbic acid. It

will also be noted in Fig. 7 that for the first few days there is a rise in the plasma ratio: $\frac{\text{Roe and Kuether value}}{\text{dye value}}$. In other experiments we have found a slow fall in the plasma dye level. This is the opposite of the effect reported by Stewart, Horn and Robson (1953), who found the ratio approximated to 1 within four hours of giving ACTH or

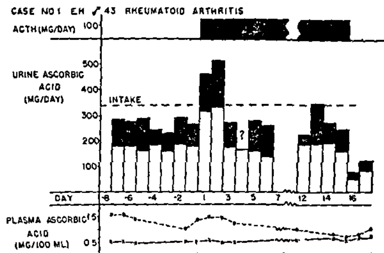
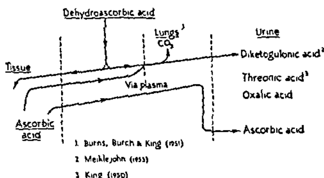


Fig. 7 ... tion in a patien' ... ascorbic acid. ... —X dye metho ... l by per-

cortisone. Beck *et al.* (1951) obtained similar results to ours, suggesting a rise in the Roe and Kuether value. They only used this method of estimation.

In view of the possible rise in the amount of oxidised material and that Long, Miles and Perry (1951) had postulated, on the basis of alterations in the sensitivity of guinea-pigs to tuberculin, that the adrenal steroids might act by increasing the oxidation of ascorbic acid to dehydroascorbic acid, further attention was directed to this problem (Clayton,

McSwiney and Prunty, 1954). From observations of the fate of dehydroascorbic acid injected into human subjects and guinea-pigs it was concluded that the relationship between it and ascorbic acid could be represented as follows:—



No evidence of the presence of oxidation products, including dehydroascorbic acid, was observed in guinea-pig tissues. Although it had been briefly stated by Long (1947) and Sayers and Sayers (1948) that the adrenal ascorbic acid measured by the dye or Roe and Kuether methods was the same after ACTH injection, it was decided to re-investigate this question in more detail. In one series of experiments the guinea-pig tissues, including the adrenal, were heavily depleted of ascorbic acid by placing the animals on a scorbutic diet for twelve days. The tissue *ascorbic acid* level was then rebuilt over a three-day period by giving in daily doses of 10 mg. and 20 mg. respectively ascorbic acid or dehydroascorbic acid by injection. During this three-day period and on the preceding day, ACTH was injected twice daily (20 units ACTHAR gel) or cortisone injected daily (5 mg.). At the end of the three-day period there was no evidence that the hormones caused the production of oxidation products in the adrenal or the liver, even when dehydroascorbic acid itself was given. *In a further experiment designed to study the period of fall* in adrenal ascorbic acid occurring during the few hours after

ACTH injection the result obtained was similar. Guinea-pigs, previously receiving an adequate diet with respect to ascorbic acid, were given an injection of 200 mg. of this substance or sodium ascorbate to flood the tissues and enable the adrenal cortex to refill during the period of ascorbic acid discharge. An injection of 60 units of lyophilized ACTH was given intraperitoneally. Fig. 8 shows that even under these conditions there was a significant fall in ascorbic acid but less than that observed by Long (1947) in spite of the very large

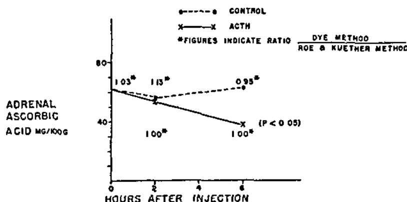
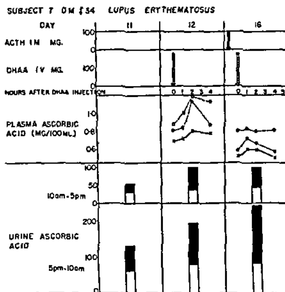


FIG. 8. Effect of ACTH (60 units soluble) on adrenal ascorbic acid in the guinea pig (for details see text)

dose of ACTH. Even though the adrenal ascorbic acid level was kept up there was no tendency for this to become oxidized in the tissue.

Although ACTH and cortisone could not be shown to produce oxidation products of ascorbic acid in the tissues, by the method employed, some evidence was obtained of an increase in oxidation products in the urine of human subjects when dehydroascorbic acid was administered. This phenomenon in not observed in guinea-pigs because when given dehydroascorbic acid they excrete primarily oxidation products in the urine and only very small amounts of ascorbic acid. In the human subjects there seemed to be an increase

in the amount of oxidation products in the urine after giving ACTH along with dehydroascorbic acid (Clayton, McSwiney and Prunty, 1954). The results of these experiments are shown in Fig. 9. Oral doses of 150 mg. of ascorbic acid were given for eleven days preceding the experiment in order to maintain good baseline excretion of ascorbic acid in the urine.



It will be seen that when ACTH and dehydroascorbic acid are injected together there is an absolute increase in the excretion of substances measured by the Roe and Kuether method. Dehydroascorbic acid itself did not form part of this increase. For a further discussion of these results reference should be made to the paper cited.

In conclusion it seems then that ascorbic acid deficiency, far from being a bar to adrenal cortical secretion, can stimulate

it intensely. There still remains a reserve capacity in thes adrenals. On the other hand, adrenal secretion does no significantly influence the development of scurvy althoug adrenalectomized animals are particularly sensitive to it Cortical hormones accelerate the mobilisation of ascorbi acid from tissues other than the adrenal and some increas in the oxidation products may occur.

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DISCUSSION

to respond

PRUNTY. We haven't investigated that point. It is worth doing.

SOFFER. Have you had any experience with the use of competitive agents of ascorbic acid on the urinary excretion of neutral 17-keto-

We could get rises of ascorbic acid excretion sometimes with cortisone.

I

that problem.

THORN: Has anyone followed the blood level of ascorbic acid with a constant infusion, and then added the hormone to see how much of this change in blood level is due to changes in renal excretion? I gather from your studies that one may have a rise at times in blood level, which suggests that increased renal clearance has not occurred. On other occasions, however, the blood level appears to fall, which would still leave both possibilities open.

PRUNTY: We have done one or two experiments in this connection, but the results are very indeterminate.

SAMUELS: Did you inject cortisone and follow the rate of its metabolism and excretion in the scorbutic subject before and after the administration of ascorbic acid?

PRUNTY: No.

SAMUELS: I think that the rise in steroid excretion which you get in

animal at this stage of any illness.

PRUNTY: There are two important points here. I would like to say we
; the
sone.
keto-
We
have not attempted to quantitate the terminal response which appears in blood level and urine excretion, with the normal response to ACTH, but it doesn't obviously seem to be a matter of utilization or divergence of metabolism. All we think we have shown is that scurvy is one factor which will set the adrenal in action. How it compares with other morbid states I don't know.

ACTH. I don't think it is necessarily related to the scurvy.

BAYLISS: At the time of death?

PRUNTY: Yes.

BROWNE: We have done 17-ketosteroid, glucocorticoid, and nitrogen

excreti
termin
I do n
very marked
nitrogen. This we did not observe in a number of other patients who were followed to death.

Soffer: I would agree with Dr. Bayliss and Dr. Samuels and Dr.

patients dying from acute leukæmia show a high spontaneous terminal or sub-terminal rise in their urinary steroid output (cf. Bernard, J., Deltour, G. H., Velez, E. and Lecomte, R., 1953, *Sem. Hôp. Paris.*, 29, 3 426)

one way or another. These secreting cells must protect themselves

in the adrenal.

In a further series of experiments in the rat (which of course synthesizes its own ascorbic acid) we looked for a possible relationship between ascorbic acid metabolism and adrenocortical function. We studied the effects of various types of stress, such as scalding, surgical trauma, and cold, as well as the effect of adrenalectomy, hypophysectomy, and administration of adrenocortical extract on the plasma level of ascorbic acid, and found in both normal and adrenalectomized animals an identical pattern of response, namely a triphasic pattern in response to surgical trauma and cold: a fall in the first thirty minutes, followed by a

pronounced rise, and then by a gradual return to normal. The response to scalding was slightly different: the animals did not show the primary fall, but only the rise and return to normal. The amplitude of the response was, however, smaller in the adrenalectomized than in the normal animals. We also found that both hypophysectomy and adrenalectomy considerably decreased the level of plasma ascorbic acid. It was, however, raised to normal or above normal levels by giving very small amounts of adrenocortical extract, which had a prolonged and maintained action (Skelton, F. R. and Fortier, C., 1951, *Canad. J. med. Sci.*, 29, 176).

In an attempt to explain the findings of Dugal, to which Dr. Prunty has referred, in relation to the inhibition by ascorbic acid administration of the adrenal enlargement consequent to chronic exposure to cold, we studied the adrenal response to ACTH following the administration of large doses of ascorbic acid. We found that while the depletion of the adrenal ascorbic acid was of lesser magnitude (which could be expected since the intake was greater), there was no change in the adrenal cholesterol response to ACTH (Skelton, F R. and Fortier, C., 1951, *Canad. J. med Sci.*, 29, 100). Dugal and Therien (*Science*, 1952, 115, 598), in
clusions.
cholester
large doses of ascorbic acid, when such treatment is continued for a period of ten days in the hypophysectomized rat. A similar conditioning effect at the post-adrenal level was recently reported by Bacchus and co-workers (*Proc Soc. exp. Biol, N Y.*, 1952, 79, 648; 80, 88), who found that the concurrent administration of cortisone and ascorbic acid to adrenalectomized mice and rats induced a greater lymphopenia, eosinopenia and liver glycogen deposition than could be accounted for by cortisone alone.

PRUNTY. We haven't done any work with the rat because of the constant synthesis of ascorbic acid, but in a few experiments with human beings some years ago, giving ACTH and ascorbic acid together, we never got any sign of potentiation of the ACTH effect.

STUDIES ON THE SODIUM-RETAINING EFFECT OF ADRENAL CORTICAL STEROIDS

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Introduction

The capacity to induce sodium retention is a property shared by a wide variety of steroid hormones, including the œstrogens, androgens, progesterone and adrenal cortical steroids. Analysis of steroids with respect to this function reveals on the one hand substances, such as testosterone, which are capable of inducing nitrogen, potasssium and phosphorus retention in conjunction with the retention of sodium and, on the other, steroids such as hydrocortisone and cortisone which facilitate the excretion of potassium, nitrogen and phosphorus whilst inducing sodium retention. Progesterone and 11-desoxycorticosterone occupy a somewhat intermediate position since they effect sodium retention in conjunction with a transient potassium diuresis, with little overall change in nitrogen and phosphorus excretion. The effects of the sodium retention which follows the administration of physiological doses of adrenal cortical hormones to adrenalectomized animals should be distinguished from those which occur with the administration of a large variety of noxious agents, including certain steroid hormones. Administration of the latter may actually shorten the survival of adrenalectomized animals It is proposed to limit the discussion at this time to a quantitative comparison of the electrolyte-regulating activity of certain adrenal cortical steroids and their derivatives.

11-Desoxycorticosterone

Desoxycorticosterone, when administered in adequate dosage to normal subjects or to patients with adrenal cortical insufficiency induces a decreased sodium and chloride concentration and an increased potassium concentration in urine, sweat, saliva and fæces (Figs. 1 and 2) (Thorn *et al.*,



1953; Conn, 1949). The overall effect of desoxycorticosterone on the electrolyte composition of gastrointestinal secretions is best demonstrated by the use of loading techniques with the concomitant administration of ion-exchange resins (Berger, Quinn and Homer, 1951).

During the administration of desoxycorticosterone, the expansion of extracellular fluid volume may well exceed the change in external water balance, indicating a shift of water

from intracellular to extracellular space (Guadino and Levitt, 1949). A significant elevation of intracellular sodium and a decrease in intracellular potassium have been demonstrated with continued high-dosage desoxycorticosterone

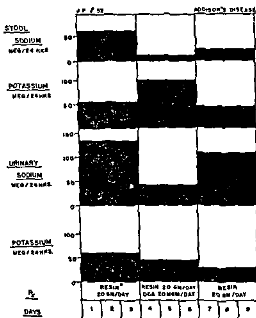


FIG. 2. Excretion of sodium and potassium in a patient with Addison's disease. The patient was on a constant diet (240 mEq of sodium and 72 mEq of potassium) Stools and urine were collected in three-day pools, and the values for sodium and potassium represent average daily excretion. (Reproduced with permission from the *New England Journal of Medicine.*)

administration to animals and man (Darrow and Miller, 1942; Fourman *et al.*, 1952).

Because of the interest to this symposium of the relationship of adrenal steroids to hypertension, the known effects of desoxycorticosterone acetate therapy will be briefly reviewed.

(1) It appears that the prolonged administration of desoxycorticosterone is capable of producing hypertension when used in the treatment of Addison's disease. For example, a forty-six-year-old white male with known Addison's disease for fifteen years exhibited a gradual increase in basal blood pressure from levels of 120/65 in 1939 to 180/100 in 1953. At the time of his last hospital admission, fundus examination revealed early hypertensive retinopathy. The electrocardiogram revealed evidence of left ventricular hypertrophy. There was no evidence of phaeochromocytoma or renal disease. The changes in this patient are similar to those reported by Perera *et al.* (1944).

(2) Aggravation of pre-existing hypertensive vascular disease has been observed in conjunction with desoxycorticosterone therapy.

A patient with pre-existing hypertension developed a normal blood pressure with the onset of characteristic Addison's disease. Following the administration of desoxycorticosterone the patient showed clinical improvement; however hypertension returned promptly and progressive retinal vascular changes were observed. Following the substitution of cortisone, 25 mg. per day, and a small supplementary dose of sodium chloride for the desoxycorticosterone therapy, the patient's blood pressure returned to normal and has remained so for three years. Complete regression of the vascular changes in the ocular fundi has occurred (Perera *et al.*, 1944).

(3) Patients whose hypertensive cardiovascular disease has been improved following bilateral adrenalectomy exhibit a hypertensive response with increased heart size following the addition of desoxycorticosterone therapy to a basic cortisone maintenance regimen.

For example: A complete bilateral adrenalectomy had been carried out on a twenty-nine-year-old white male for severe hypertension characterized by a basal blood pressure of 230/170 mm. Hg., cardiac enlargement, grade III hypertensive retinopathy and albuminuria. Postoperatively the patient

had been maintained on cortisone alone in a dosage of 25 to 50 mg. per day. On this regimen the basal blood pressure [illegible]

the heart size increased to 27 per cent above normal and the blood pressure values exceeded 160/100 mm. Hg. At this point it was felt imperative to reduce the desoxycorticosterone dosage to 1 mg. per day. On this schedule the heart size decreased to 6 per cent above normal and the blood pressure fell to a level of 120/80 mm. Hg.

(4) It is generally accepted that retention of sodium is essential for the production of hypertension by desoxycorticosterone acetate. Furthermore, in experimental animals reduction in renal functioning mass appears to facilitate this change (Selye, Hall and Ranley, 1943). Studies on patients with kidney disease treated with desoxycorticosterone acetate support these experimental observations (Thorn *et al.*, 1952). In order to test the hypothesis that sodium retention is essential for the production of hypertension in the presence of renal disease, a patient with salt-losing type of nephritis was selected for study. This type of patient is known to be unresponsive to the sodium-retaining effect of desoxycorticosterone because of end-organ failure. This patient was given a large dose (30 mg. daily) of desoxycorticosterone acetate intramuscularly for a period of four to six weeks without evidence of sodium retention and without a significant alteration in blood pressure. The failure to observe hypertension following the prolonged administration of desoxycorticosterone in this patient suggests the importance of renal mechanisms, especially salt retention, and the less important place of extrarenal vascular change in the absence of salt retention.

Corticosterone and Dehydrocorticosterone

The presence of corticosterone in a relatively high concentration in adrenal extracts has long been recognized (Thorn

et al., 1953). Its significance is again emphasized by the studies of Hechter *et al.* (1951) who have shown that corticosterone and 17-hydroxycorticosterone (hydrocortisone) are the principal steroid substances obtained by perfusion of the isolated bovine adrenal gland with ACTH. Furthermore, chromatographic studies on human adrenal venous blood indicate that the quantity of corticosterone recovered is second only to that of hydrocortisone (Romanoff, Hudson and Pincus, 1953). As might be anticipated from the chemical structure of corticosterone and dehydrocorticosterone, these steroids exhibit qualitatively the electrolyte-regulating activity of desoxycorticosterone in addition to the carbohydrate-regulating activity of cortisone and hydrocortisone. The electrolyte-regulating effect of corticosterone can be demonstrated with oral, as well as parenteral, hormone administration. In this respect, corticosterone differs appreciably from desoxycorticosterone, which is relatively inactive when given by mouth (Thorn *et al.*, 1953). Conn's studies (Conn *et al.*, 1951*a*) and the authors' would indicate that the intramuscular injection of corticosterone is two or three times as effective as oral administration.

Attempts have been made to utilize corticosterone as the sole replacement therapy in Addison's disease. The relatively high sodium-retaining potency of this compound limits its usefulness in most patients since optimum carbohydrate regulation cannot be attained without the development of œdema or other evidence of excessive sodium retention. Patients with a relatively high requirement for salt-retaining hormone, however, can be maintained in excellent condition for prolonged periods by corticosterone administered either orally or by intramuscular injection. The limited quantity of material available at present and the relatively high cost have precluded any extensive clinical evaluation.

The position which corticosterone occupies as a sodium-retaining hormone is illustrated by the response of a completely adrenalectomized hypertensive patient who showed enlargement of the heart and restoration of hypertension with

a maintenance dose of desoxycorticosterone (2 mg.) or corticosterone (50 mg.), but not with a maintenance dose of cortisone (25 mg.).

The potassium diuresis which occurs in patients treated with corticosterone is minimal and transient. It more closely resembles the potassium diuresis observed with desoxycorticosterone administration than that which is seen with cortisone or hydrocortisone. This fact, coupled with the minimal changes in nitrogen excretion, suggests that corticosterone is relatively deficient in protein anti-anabolic activity.

11-Desoxy-17-hydroxycorticosterone (Compound S)

This substance, which was first isolated in 1938 from beef adrenal by von Euw and Reichstein and subsequently synthesized by these investigators in 1940, exhibits only weak salt-retaining activity. Clinical studies indicate that quantities as large as 200 mg. per day must be administered to induce detectable changes in sodium chloride balance.

11, 17-Oxygenated Corticoids

Cortisone (Compound E) was first isolated from adrenal cortical extracts in 1936, almost simultaneously in the laboratories of Kendall (Mason, Myers and Kendall, 1936), Reichstein (1936) and Wintersteiner and Pfiffner (1936). Partial synthesis of cortisone from desoxycholic acid was eventually accomplished in 1946 by Sarrett (1946), employing methods extending the procedures developed by Reichstein and Kendall.

Hydrocortisone, or Compound F, was first isolated from adrenal cortical extracts in 1937 by Reichstein (1937) and by Mason, Hoehn and Kendall (1938). In 1948, Mason and Sprague isolated hydrocortisone from the urine of a patient with Cushing's syndrome. Subsequently hydrocortisone has assumed increasing importance with the discovery that it is the principal steroid in adrenal gland perfusates and in adrenal venous blood and peripheral blood. These findings have led to the view that hydrocortisone is the principal hormone

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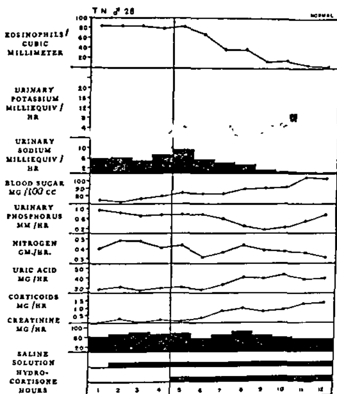
this hormone at the rate of 12 mg. per hour (Fig. 3). The sodium-retaining effect became apparent about two hours after the beginning of the infusion and reached maximum at six hours. It is to be noted that the sodium excretion in the urine fell from an initial control level of 6 mEq. to 0·5 mEq. per hour. In contrast to this, tetrahydrocortisone, an important metabolite of hydrocortisone, produced no significant change in sodium excretion when given intravenously at the same rate.

The intramuscular injection of 300 mg. of hydrocortisone free alcohol daily resulted in a retention of sodium almost as marked as that observed following the intravenous infusion of this hormone. However, intramuscular injection of hydrocortisone *acetate* exerted only a weak sodium-retaining effect at this high dose level! The relative therapeutic inefficacy of the acetate of hydrocortisone administered intramuscularly has been well substantiated (Boland, 1952). When hydrocortisone acetate is administered orally rather than intramuscularly it is more effective in producing sodium retention and the difference between the free alcohol and the acetate is less marked (Boland, 1952; Conn *et al.*, 1951*b*).

It has been noted from time to time that increased sodium excretion occurs, particularly in the initial phases of hormone administration. Twenty-four-hour renal clearance studies carried out by Finkenstadt and Dingman (1954) in our laboratory, with hydrocortisone given intravenously at the rate of 10 mg. per hour, demonstrated an initial increase in sodium excretion, prior to sodium retention, in normal subjects and Addisonian patients (Figs. 4 and 5). However, when hydrocortisone was given at the rate of 1 mg. per hour intravenously to patients with Addison's disease, sodium diuresis persisted throughout the twenty-four-hour period. The sodium diuresis appeared to be the resultant of a relatively greater increase in glomerular filtration than in tubular reabsorption of sodium, while the subsequent sodium retention with the larger dose of hydrocortisone resulted from further enhancement of tubular reabsorption.

secreted by the human adrenal cortex. Since the metabolic effects of cortisone and hydrocortisone are similar, the present discussion will be limited to hydrocortisone.

The effect of hydrocortisone on electrolyte excretion in



man is more variable than that observed with either 11-desoxycorticosterone or corticosterone. The magnitude of sodium retention varies with the dose and type of hormone preparation and the route and duration of administration.

Maximum sodium retention with hydrocortisone has been demonstrated following the intravenous administration of

cortisone. It was anticipated that the patient's tubular reabsorption of sodium would not appreciably be affected because of end organ failure. It was of interest to note that in association with the continued intravenous infusion, over

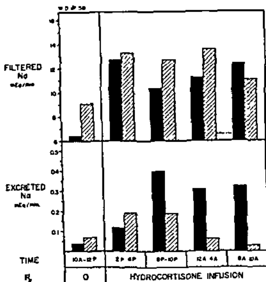


FIG. 5. Effect of continuous intravenous hydrocortisone on renal filtration and excretion of sodium in Addison's disease.

■ *Hydrocortisone 1 mg /hr.*
⁄ Hydrocortisone 10 mg./hr.

Filtered sodium refers to serum sodium times the rate of glomerular filtration, determined by the inulin clearance technique. Sodium reabsorbed equals filtered sodium minus excreted sodium.

twenty-four hours, of 250 mg. of cortisone daily for two days the serum sodium level rose and the urinary excretion of sodium increased, despite a constant sodium intake.

That the reverse of the above stage may occur in the absence of adrenal cortical hormones is indicated by the studies of Arons and Nusimovich (1954). These studies were

An important effect mediated by the 11, 17-oxygenate steroids is the restoration toward normal of the elevate intracellular sodium and extracellular potassium which ar characteristic of many serious and chronic disease states Under these circumstances it is postulated that followin

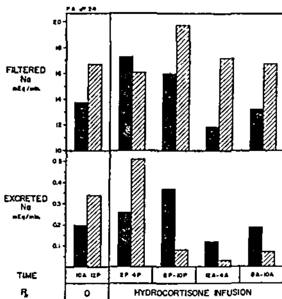


FIG. 4. Effect of continuous intravenous hydrocortisone on renal filtration and excretion of sodium in a normal subject.

■ Control ⁄⁄ Hydrocortisone 10 mg./hr.

Filtered sodium refers to serum sodium times the rate of glomerular filtration, determined by the inulin clearance technique. Sodium reabsorbed equals filtered sodium minus excreted sodium.

the administration of large doses of cortisone or hydrocortisone a shift of water and electrolytes occurs, with the overall result that the sodium content of the extracellular fluid compartment is increased, thus increasing the load of sodium presented to the kidney.

This phenomenon has been observed in a patient with "salt-losing nephritis" given an intravenous infusion of

account for a failure to observe a negative sodium balance in this patient despite a marked reduction in adrenal cortical secretion. Thus, the incapacity of the tubules to reabsorb sodium effectively could be compensated in part by reduced glomerular filtration due to dehydration and hypotension and by a diminished sodium concentration in the glomerular filtrate as a result of a shift of sodium from the extracellular compartment to the cells. Thus, the paradox of low serum sodium and high serum potassium in the presence of normal body exchangeable sodium and potassium may be explained by this mechanism.

Thus it can be seen that in considering the mode of action of adrenal cortical hormones on electrolyte metabolism one must consider an effect not only on renal function but on the distribution of sodium and potassium between the intracellular and extracellular compartments.

Halogenated Derivatives of Hydrocortisone

Fried and Sabo (1954), while attempting to synthesize 17α-hydroxycorticosterone from 11-epi-17α-hydroxycorticosterone via a halogenated intermediate, found that the 9α– halogen derivatives of hydrocortisone were highly active in the rat liver glycogen assay for 11-oxygenated corticosteroids. Borman, Singer and Numerof (1954) studied the halogenated derivatives of hydrocortisone with respect to their life-maintaining, sodium-retaining and glycogen deposition properties in rats. Their findings indicated that fluorohydrocortisone was less active than desoxycorticosterone in growth and weight maintenance, eight times as active as cortisone acetate in liver glycogen deposition and twice as active as desoxycorticosterone in salt retention.

The comparative potency of hydrocortisone acetate, chlorohydrocortisone acetate and fluorohydrocortisone acetate has been studied in a patient with Addison's disease maintained on a constant regimen (Fig. 7). The introduction of a chlorine or fluorine atom at the carbon-9 position not

carried out on a patient in severe Addisonian crisis. During the three months prior to admission the patient had shown progressive weight loss, anorexia, nausea, vomiting, dehydration and hypotension. The diagnosis of Addison's disease was established by the clinical findings, the absence of 17-hydroxycorticoids in the urine and by the failure of the adrenal to respond to three days of intravenously adminis-

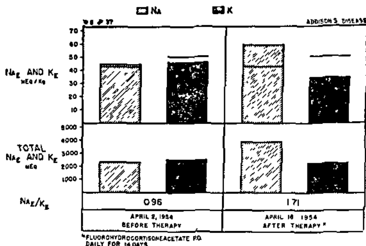


FIG. 6.
body sod
sodium,
detern

tered ACTH. Somewhat unexpected was the finding that the exchangeable body sodium and potassium in this patient were well within the normal range. The ratio of these two quantities was likewise normal (Fig. 6). These findings, together with the observation of a low serum sodium concentration, an elevated serum potassium, and clinical evidence of dehydration, strongly indicate that with the progress of acute adrenal insufficiency there was a gradual decrease in intracellular potassium and an increase in intracellular sodium. These changes suggest a mechanism which could

hormones in a second patient with Addison's disease maintained in electrolyte balance on a constant diet and 25 mg. of hydrocortisone acetate by mouth daily, indicated that almost identical effects on sodium and potassium excretion were attained with desoxycorticosterone, 2 mg. by intra-

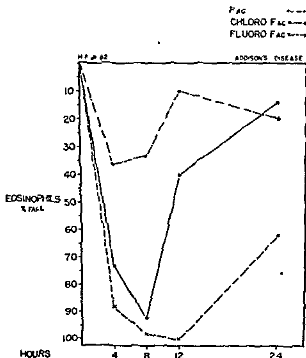
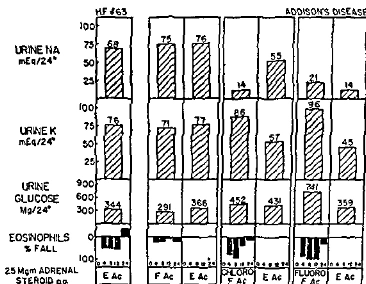


FIG. 8. Eosinopenic response to 25 mg. orally of hydrocortisone acetate, chlorohydrocortisone acetate and fluorohydrocortisone acetate.

muscular injection, and with either 6 mg. of chlorohydrocortisone acetate or 0·5 mg. of fluorohydrocortisone acetate administered in a single dose by mouth.

There appears to be appreciable sodium retention, in some patients at least, with a single daily dose of 250μg. of fluorohydrocortisone *by mouth* (Fig. 9). This effect corresponds

only intensified the salt-retaining potency of hydrocortisone, but measurably extended the period of activity. The eosinopenic action was proportionately enhanced (Fig. 8). Analysis of the activity of these derivatives indicates that not only is the fluorohydrocortisone much more potent in its sodium-



was replaced with the test compounds which were given in a single oral dose of 25 mg. per day.

retaining effect than any of the other forms of hydrocortisone tested thus far, but that it is also exceeds by several-fold the sodium-retaining effect of desoxycorticosterone—the latter being given intramuscularly as compared to the *oral* efficacy of fluorohydrocortisone. It is unique in this instance that this form of hydrocortisone exhibits a more striking sodium-retaining effect than the classical sodium-retaining hormone desoxycorticosterone acetate. Comparative studies of these

oral use, and to Dr. Harold F. Hailman of the Upjohn Company for hydrocortisone free alcohol and hydrocortisone acetate for intramuscular use.

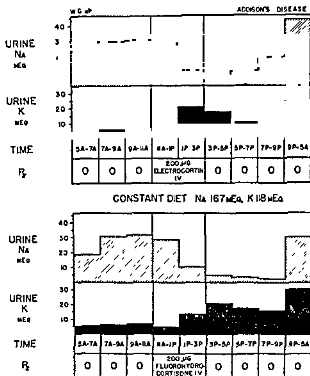


FIG. 10 A comparison between the effect of intravenously administered electrocortin (aldosterone) and fluorohydrocortisone on the urinary sodium and potassium excretion.

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closely to that observed by Mach *et al.* (1954) when the same total quantity of electrocortin (aldosterone) was given in divided doses every six hours by intramuscular injection. In one patient with Addison's disease it has been possible to compare the sodium-retaining effect of electrocortin and fluorohydrocortisone, the material in both instances being given as a continuous intravenous infusion over a period of

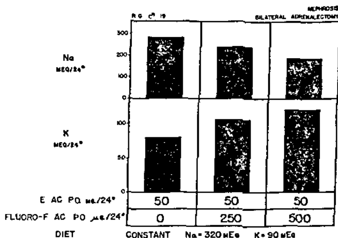


FIG. 9. The effect of fluorohydrocortisone on urinary Na and K.

four hours (Fig. 10). It is to be noted that the intensity of the sodium-retaining effect was almost identical during the infusion. However, the more sustained effect of fluorohydrocortisone is readily apparent in the six-hour period following the termination of the infusion.

Acknowledgements

of Merck and Company, Inc. for hydrocortisone for intravenous and

CLINICAL AND METABOLIC EFFECTS OF ALDOSTERONE

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IN the first part of this symposium, Simpson and Tait, as well as Gaunt, showed the results of their experimental work with aldosterone. Today we would like to give you our first impression of the clinical and metabolic effects of aldosterone.

In February 1954 our group began the first metabolic study on two patients with Addison's disease who received small doses of aldosterone (see Table I). In a second study, we tried

Table I

CLINICAL AND METABOLIC STUDIES WITH ALDOSTERONE

(a) [illegible]

2nd. case: ♂, thirty-five years, Addison's disease of five years duration, last implantation eleven months ago.
Three days control
Five days of aldosterone (150, 150, 200, 200, 200 $\mu g.$)
Two days with 4 mg. DCA q d.

(b) *Subjects without adrenal insufficiency*

1st. case. ♀, seventy-four years, Rheumatoid Arthritis, hypertension, low sodium diet (1–2 g)
Two days control
Three days of aldosterone (500, 300, 300 μg)
Two days control

2nd. case. ♂, twenty-three years, medical student, high sodium intake
Four days control
Three days of aldosterone (1000, 750, 750 $\mu g.$)
Four days control

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[Discussion of this paper was postponed until after the paper by Dr. Mach.—*Ed.*]

All the symptoms of adrenal insufficiency reappear seven or eight hours after the last injection, no matter what dose is given. We were therefore able to determine with precision the *limits and the duration of the therapeutic action* of our preparation, which led us to distribute the injections of aldosterone at regular intervals three times a day.

In spite of the shortness of our experiments, they enable us to make an important observation. In both subjects with Addison's disease, aldosterone produced a rapid and unexpected effect on the pigmentation of the skin. In the first patient, for instance, the effect of aldosterone on the pigmentation was much more marked after six days of treatment, than of cortisone administered for several months.

In spite of an evident clinical improvement and an increase of the blood volume, the arterial pressure of the two patients was even slightly lower during the administration of aldosterone than during the period of adrenal insufficiency. This fact is even more significant when it is noted that DCA caused a marked increase of the arterial pressure in the same two patients, surpassing normal values. The first subject, who generally runs a pressure of 160/110, never developed hypertension under aldosterone treatment. This observation permits us to establish an interesting difference in the action of the two hormones on the vascular tone, which if it were found in a greater number of cases, would have important therapeutic consequences.

A very significant point, which is also brought out in the study of these two Addisonians, is the fact that relatively high doses of aldosterone, which bring about complete clinical recovery, do not produce pathological retention of water. This observation is in accordance with the findings of Gross in experiments on dogs. He also showed that the water retention caused by DCA will disappear following the administration of aldosterone.

Aldosterone is a natural hormone, and it seems to correct arterial hypotension and electrolyte disturbances within physiological limits. This is a most important biological fact

to demonstrate the metabolic effects on two subjects without adrenal insufficiency. To do so, we were obliged to use large doses of aldosterone.

The first patient, who had had Addison's disease for three years, was kind enough to permit the removal of his DCA pellets in order that we could accomplish our study with aldosterone. We maintained him on 6 mg. DCA a day for two weeks before the onset of the experiment. As you see in the first table, we divided the study into five different metabolic periods.

In Table II it is evident that all the symptoms of adrenal insufficiency—fatigue, nausea, anorexia, sleepiness, depres-

Table II
CLINICAL ACTION OF ALDOSTERONE

Before treatment		*During treatment*	*After treatment*
Addison's disease	Fatigue Nausea Anorexia Sleepiness Depression Dyspnœa on effort	Disappear one hour after the injection	Reappear 7–8 hours after the last injection
	Ergogram (15 kg./sec.) 84 sec	280 sec.	
	Pigmentation		diminution already after 5 days of treatment
Rheumatoid Arthritis	Classical signs, Hypertension	without effect	without effect

sion, dyspnoea on effort—disappear one hour after the first injection. During the treatment, the two patients felt as well as during a combined treatment of DCA and cortisone. The first patient, who is very intelligent, noticed a feeling of alertness and even happiness. The correction of asthenia, as measured by the ergogram, shows the remarkable effects of aldosterone.

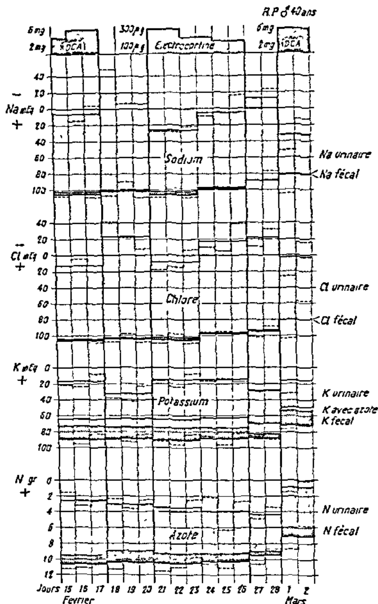


FIG. 1. Addison's disease, first case. Balance of electrolytes and nitrogen.

which doubtless would be of great advantage in the treatment of Addison's disease.

Metabolic effects

During the experiment we maintained the patient on a constant diet and kept an exact balance of chloride, sodium, potassium, nitrogen, calcium and phosphorus intake and output. As you see in Fig. 1, aldosterone produces in patients with Addison's disease a retention of chloride and sodium, and an increase in the excretion of potassium. During the first three days of treatment our subject retained a total of 64 mEq. of sodium and 72 mEq. of chloride. The potassium excretion was influenced in the characteristic way; its increase in the urine, which appeared after the first injection of aldosterone, is very clearly illustrated in Fig. 1. Fig. 2 shows the remarkable effects of aldosterone on sodium and water metabolism in a second case of Addison's disease.

Now let us examine the metabolic effects of aldosterone in subjects without adrenal insufficiency. The first case, a female patient with rheumatoid arthritis and hypertension (Fig. 3), had received a very low sodium diet for several months. In spite of the high dose of aldosterone (400 μg. a day), she retained a very small amount of sodium, but had a characteristic excretion of potassium. We observed no effect on the clinical symptoms of rheumatoid arthritis, on the sedimentation rate or on the number of eosinophils in the blood.

The next experiment was on a normal medical student, who was willing to undergo this study on condition that he had a high caloric intake. This explains the considerable amount of sodium in his diet (Fig. 4). This subject, in contrast to the previous one, shows a marked retention of sodium and chloride, but no change in the potassium excretion. We think that the difference in the response of these two subjects on high doses of aldosterone may be explained by the different composition of their diet. We would like to emphasize that it is impossible to judge the effects of a

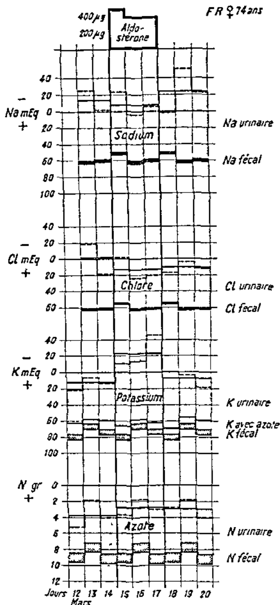


FIG. 3. Rheumatoid arthritis. Balance of electrolytes and nitrogen.

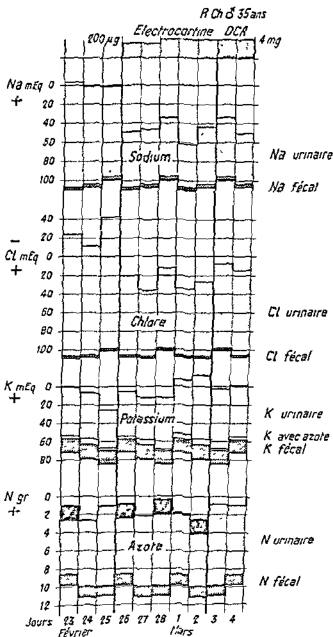


FIG. 2. Addison's disease, second case. Balance of electrolytes and nitrogen

hormone on electrolytes unless the composition of the diet is known in all details. There was a minor accident in the balance study of the medical student (*Fig.* 4). *In order to do* a glucose tolerance test, the subject fasted the whole morning and to keep up with his food intake he ate too much at four o'clock; he developed gastro-intestinal disturbances and for this reason cut down on his food intake in the evening. The reduction of the sodium intake is actually not so important,

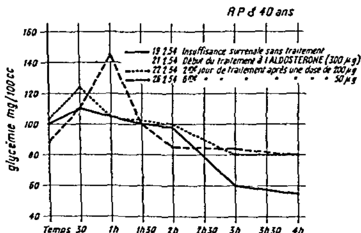


FIG. 5. Action of aldosterone on glucose tolerance test in the first subject with Addison's disease.

when one realizes that the 40 mEq. lost make up one-eighth of the total sodium intake.

In this case, it is important to point out the psychological changes with aldosterone. The subject had a feeling of alertness and was able to work very hard for several hours; it was a typical cortisone-like effect.

The influence of aldosterone on the carbohydrate metabolism was of great interest to us. Fig. 5 illustrates the action of aldosterone on the glucose tolerance test in our first subject with Addison's disease. The classical curve, initially flat followed by a sustained hypoglycæmia (before treatment)

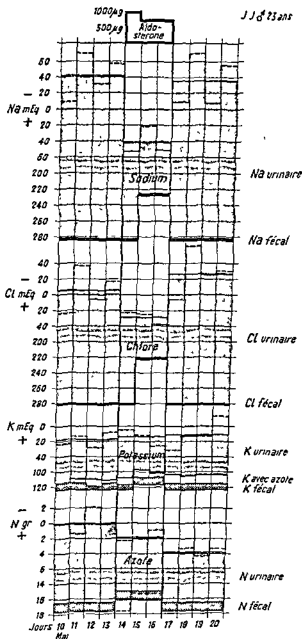


FIG. 4. Normal subject. Balance of electrolytes and nitrogen.

Table III

EOSINOPHILS PER MM³

	Aldosterone	8 *hr.*	12 *hr*	18 *hr.*
10·3	—	305	—	—
11 3	—	—	—	—
12 3	—	305	285	—
13 3	—	—	—	—
14·3	500+250+250 µg.	305	162	155
15 3	500+250 µg.	155	—	—
16 3	3×250 µg.	—	—	—
17 3	—	295	235	269
18 3	—	363	—	—
19·3	—	—	—	—
20 3	—	338	—	—

Summary

Following a period of adrenocortical deficiency without treatment, two cases of Addison's disease have been treated with the new adrenocortical hormone aldosterone (electrocortin). The clinical syptoms of the disease disappeared within a few hours after the beginning of the treatment. Within a few days, skin pigmentation regressed, and the orthostatic blood pressure regulation was restored without abnormal increase of the arterial pressure.

Like desoxycorticosterone acetate (DCA), aldosterone caused Na and Cl retention and increased K excretion; in the blood, the typical signs of dilution were noted, as well as slight decrease of the K level.

The effect of aldosterone on carbohydrate metabolism was evident from the normalisation of the blood sugar curves in the glucose tolerance test; the hyperglycæmic peak was increased, the hypoglycæmic reaction suppressed.

One of our patients suffered from arthralgia in both knees and this disappeared during aldosterone treatment.

In a case of severe rheumatoid arthritis, without any symptoms of adrenocortical deficiency, aldosterone during three days (500 µg., 400 µg. and 300 µg. per day) had no effect

was modified with aldosterone; the blood sugar rose to 126 mg. per cent and then to 150 mg. per cent and we observed the correction of the hypoglycæmia reaction; in other words, the glucose tolerance test became normal. In the second patient with Addison's disease the action of aldosterone is not evident, because the curve was not really pathological to

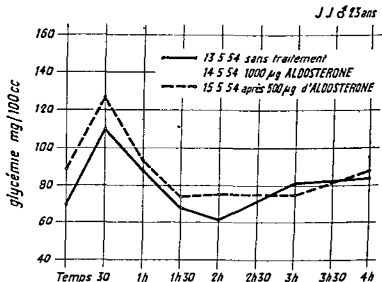


FIG. 6. Glucose tolerance test in a normal subject before and during aldosterone therapy.

start with. The glucose tolerance test of our medical student was interesting because we observed a real improvement (Fig. 6).

Besides the positive observations, we made a series of negative observations which we can summarize briefly:

(1) *Aldosterone exerts no action on the nitrogen balance.*
(2) Aldosterone does not improve the water load test.
(3) Aldosterone in doses of 200 to 300 µg. a day does not change the number of the eosinophils in the blood. In using larger doses of 1000 µg. a day we observed in one normal subject so far studied the typical fall in the eosinophils (Table III).

shown when one has enough material to compare the effects of both compounds in the same patient. We have studied the carbohydrate

particularly good compound for anti-inflammatory reaction because of the relative excess of salt retention over carbohydrate regulation.

Mach. Our two patients with Addison's disease were not in such a severe state as yours, Dr. Thorn. They were conscious, sleepy, with stomach troubles, but not quite in Addisonian crisis.

Soffer: We have treated one patient with Addison's disease with aldosterone for a period of nine days. We had approximately 1 mg of the material available. As Dr. Mach has described, there was very little change in blood pressure during this nine-day period, employing 100 μg. of the material per day. There occurred some increase in the extracellular fluid compartment but no appreciable change in the total body water Incidentally this is a phenomenon which we have observed with cortisone as well as with DCA, and I think in part at least it represents a shift of fluid from inside the cell to the extracellular compartment This is corroborated by the fact that there occurred no

change in the water-loading test, and no alterations in carbohydrate metabolism.

Bayliss: How about pigmentation?

on the clinical picture of the disease, producing, however, the well known metabolic alterations. It is only with doses of 1000 μg. per day that we observed a fall of the eosinophils exceeding 50 per cent.

At the low doses used in this investigation, aldosterone exerted no effect on the formed elements of the blood, on nitrogen metabolism, on the water-load test, and on the 17-ketosteroid and formaldehydogenic corticoid levels in the urine.

In the subjects suffering from Addison's disease, the effective dosage was between 150 and 200 μg. aldosterone per day (2·5–3·3 μg./kg.). Thus, aldosterone was 20–30 times more potent than DCA. Unlike DCA, aldosterone does not seem to cause elevated blood pressure or pathological water retention.

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DISCUSSION

FORSHAM: Could Dr. Thorn give us any more information on the comparison of aldosterone and fluorocortisone?

THORN: We had only 1 mg. (approx.) of aldosterone to work with. We gave some to a patient in whom we compared the effects of 9 α-fluoro-hydrocortisone and aldosterone (Fig. 10). He was in severe Addisonian crisis, had never been treated before, and had lost a tremendous amount of weight. He was given 100 μg. of aldosterone every six hours by injection, but there was no striking change in his clinical condition in the first twenty-four hours We weren't able to measure sodium excretion beforehand Certainly later on the aldosterone and subsequently on the fluorohydrocortisone the excretion of sodium was very low. I don't think there is any doubt that there was an effect, but at the 400 μg. (per twenty-four hours) level in this particular patient the effect was not striking for the first twenty-four hours. We were only able to carry him for the one day. I think that probably a very seriously ill patient might need an appreciably larger dose of material in the first twenty-four hours.

So far as I can judge, the salt-retaining effect of these two compounds is of roughly the same order of magnitude; some difference may be

series of events follow. In the first place, there occurs a very considerable increase in the extracellular compartment as measured by both inulin and thiosulphate. (Incidentally we compared inulin and

administered In a general sense one may say that on a limited sodium intake in the presence of normal renal function an inverse relationship exists between the degree of expansion of the extracellular fluid compartment present and the salt- and water-retaining effect of the adrenal cortical fractions

hormone was to promote a diuresis of sodium. Dr. McCracken in my laboratory has done a series of experiments with 500-ml infusions of 5 per cent saline over a period of thirty minutes. Under that regime you get a very rapid increase in extracellular volume, and a rise in serum sodium level of about 10 m Eq. During these experiments we infused hydrocortisone intravenously, and we put the saline in at a time when

SOFFER: I could see no significant change in pigmentation, although the patient spontaneously commented that he thought his nose was getting a little lighter in colour.

GROSS: I should like to refer to our water-load experiments. With a

concentration of sodium in the blood goes up. Dr. Soffer, you found there had been *some increase in extracellular fluid. Did you plot* sodium concentration?

SOFF

GROS

down,

dose there is no increase.

BROWNE: Then you get *a shift of course. In certain brain injuries*

combined with 50–100 mg. of cortisone. The EEG did not come back to normal in our patient on a small maintenance dose of the fluoro compound, and the water-loading effect was still impaired. As for the

WILKINS: Dr. Thorn, have you found any changes in steroid excretion or blood steroids with the fluoro compounds?

THORN: With a single dose by mouth of 5 mg. of the fluoro compound we found a marked depression in ketosteroid excretion.

BROWNE: Dr

and then trans

excretion. I th

replacement of a relatively high sodium-retaining substance on the kidney tubules by one of lower sodium-retaining activity. What would you feel about that now in terms of your discussion regarding the glomerular filtration?

THORN: My belief (without proof) would be that practically every patient on DCA has excessive salt retention, and if we add cortisone we may initiate an internal shift of electrolyte and water. We felt earlier that the increased Na excretion might reflect competition between hormones, but as one *carries out successive experiments one does not*

these cases seems to have a tendency to promote salt loss rather than

Restoration of normal cellular potassium results in expulsion of the sodium that has leaked in. During the past year we have been looking for electrocortin-like substances in the urine, and the only patient in whom we found very high concentrations had no abnormality of sodium metabolism at all, but had been flown to this country from Africa because of an extreme loss of potassium leading to muscle paralysis.

just when you do these things?

Thorn. One must compromise in such a study right at the beginning. The closest approximation that we can make is to establish an arbitrary diet before operation, study the patient's balance on that diet, and then subsequently put him back on the same diet following operation and carry out similar studies. This excessive sodium loss will taper off very rapidly if left uncontrolled, provided the patient doesn't develop adrenal or circulation collapse. Later on I think there are secondary adaptation effects. One must remember that before operation the patient is œdematous, and the constant diet is constant diet plus a load.

Prunty: Yes. But it does rather depend on the conditions on the day of operation.

Thorn: The degree of loss does, yes.

Luft We have been interested for some years in the effects of DCA on electrolyte and water balance. In all our patients when we continue DCA administration we get a loss of sodium after some time, but at the same time the total body sodium increases. This means to me that there is a mobilization of sodium from some depots in the body.

We have also been able to confirm Dr. Soffer's findings on the correlation between the inulin space and inulin clearance, in healthy people and also in different endocrine disorders. There seems to be a straight-line correlation between inulin space and clearance. It is

we figured the hydrocortisone infusions were maximally intense. In one patient we observed a considerable reduction in sodium excretion under this regime; in another we got a most intense excretion of sodium, the other patients seemed to fall in between. That merely reinforces one's impression that the factors involved in sodium excretion are rather complicated. We know we raised the serum level, we know we had a maximal hormone action, and even then the response is very variable.

circumstances that small amounts of hydrocortisone would give you sodium excretion, you might be dealing with the effects on the renal tubule of the replacement of a strong endogenous sodium-retainer (e.g. aldosterone) by a weaker one Woodbury's work on shock thresholds in rats demonstrates a very definite antagonism of DCA and hydrocortisone in which hydrocortisone increases sensitivity to electric shock and DCA opposes it. And there are other direct antagonisms at the cellular level.

BROWNE: What about Dr Fourman's experiment with regard to obtaining in a single individual first sodium retention and then excretion? As far as I know this was a normal person, and no marked change occurred in his condition. Secondly, with regard to Dr. Thorn's suggestion that there is increased glomerular filtration rate and excretion and that the tubules are knocked out: in a case of renal shut-down, after the first phase one often gets a salt-losing syndrome, and I wonder whether cortisone is likely to produce a further loss of sodium.

THORN: We have tried that in two patients with Addison's and renal

[illegible]
diuresis with cortisone in a nephrotic patient, but we weren't able to show much. I think the difficulty is that we were unable to obtain constant enough circumstances All we can say is that the result in any given situation is almost unpredictable, except that if I knew a patient was on DCA or was œdematous, I would expect that cortisone would be likely to cause initial sodium diuresis.

patients. [illegible]
the ketosteroids rise and they lose salt. The pattern of hormones in

doses of DCA
ruption, to a
cases presented
hypertension was observed to disappear when the patient was placed on a lower dosage of DCA.

THORN: All the patients we have seen so far who developed hypertension were reversible by changing the dose of DCA or substituting cortisone. Perhaps Dr. Forsham would report his experience.

FORSHAM: The experiments of Selye that were referred to were done on rats. They received high doses of DCA for about two weeks and were then left to develop malignant hypertension and periarteritis nodosa. In human beings I have never seen a case of periarteritis develop with the small doses of DCA used

DELTOUR: According to the observations of de Gennes in 1948 in our Department we think there are two types of hypertension produced by DCA administration to patients with Addison's disease. One type is a

large amounts of DCA in the form of pellets. Most of these patients were observed over four to six years; in some of them, each time the DCA resorption decreased, their hypertension decreased too, but in some cases it did not, at least for a long time. Some of these patients

Addison's disease developing "dry" hypertension and rheumatic disorders, and in those who did not show any such side effects (Deltour an

F

haven't done very many cases.

FORSHAM: If aldosterone is given in very large quantities like 1–3 mg *for a week, will the blood pressure go up? It seems to me that this might* be the crucial difference between deoxycorticosterone and aldosterone.

FORSHAM: Dr. Selye postulated that growth hormone affected the mineralocorticoid excretion via the adrenal. However, Dr. Leslie Bennett, at the University of California in Berkeley, has shown that adrenalectomized rats
given growth hormone.
retainer and the adrena
at all.

THORN: Dr. Luft's remarks bring up a point we have been interested in for some time; if one does careful balance studies and gives cortisone, one can obtain a discrepancy between the quantity of sodium measured by balance technique and that measured by exchangeable sodium technique. I think there is good evidence and his experiments would

SOFFER: The decrease in the extracellular compartment which may follow the administration of the adrenal steroids may not always be

extracellular compartment into the cell and to other depots such as bone.

THORN: I am most interested in Dr. Luft's suggestion that there may be a relation between growth hormone and the mineralocorticoids. Dr. Mach, would you care to comment on that?

MACH: W
sterone treat
in proteins,
did Dr. Soffer. This is a shift between extra- and intra-cellular fluid.

high doses of aldo-

, an assay for
n of sodium in
ted by growth
hormone. It was true that the radioactive sodium in a very short period accumulated much more in the cartilage then it did in the rest of the bone; consequently there is some degree of fairly rapid exchange-

probably true also for the carbohydrate effect, although that is more difficult to measure.

LONG: Does chlorocortisone induce salt retention in any species?

THORN: It does in man, but I have had no experience with it in animal studies.

FORSHAM: Liddle, Barrter and Pechet in Washington have produced

cortisone only in degree.

*CALLOW, R. K., LLOYD, J. and LONG, D. A. (1954). *Lancet*, ii, 20.

THORN: It will take a long time to tell, because patients vary tremendously in their blood pressure response.

BERGENSTAL: With regard to the effects of DCA in Addisonians and in adrenalectomized patients, on very high doses of DCA alone we observed

simply gave him 50 mg. cortisone, in twenty-four hours the stiffness had entirely disappeared. If I took him off the cortisone it returned again. I tried giving potassium, as I think Dr. Thorn did, and was unable to alter it at all.

THORN: I think Dr. Cope's suggestion that potassium is important, was a good one. We have done a parallel study in another uræmic patient with a low serum sodium and a B.U.N. of 150. We kept the patient on a very rigidly restricted sodium intake, and with the addition of potassium produced a shift in sodium, i e a rise in serum sodium and an increased sodium excretion. In that respect I think the hormone action and the administration of potassium are quite similar.

BAYLISS: Doesn't one see the same thing in patients with congestive heart failure who develop hyponatræmia? A proportion of those respond to potassium.

THORN: I would agree that we have no evidence that the effect of the hormone requires a potassium shift before one obtains a change in sodium metabolism, but there is no doubt that an alteration in

body, but not these dramatic initial changes that you observe in the

cortical hormones without intermediation of the kidneys. On the other hand, it seems reasonable to assume that the rapid changes in the excretion of fluid and electrolytes, when patients on a normal sodium intake are given DCA, are connected with changes in the reabsorption of sodium and chloride in the renal tubules

LONG: Does the biological activity of fluorocortisone differ from that of chlorocortisone only in degree?

THORN: As far as sodium retention in man is concerned, yes. That is

rate of flow affects the saliva composition, but the relation of flow to Na and K concentration in human saliva has not been fully described. Fig. 1 gives the values of a pair of saliva samples from each of 33 healthy young men plotted against flow. The Na and Na/K values increase proportionally with flow while the K values appear to be independent of flow.

Sex and Age. Fifty-one samples from female adults and *33 samples from children under twelve years of age have been*

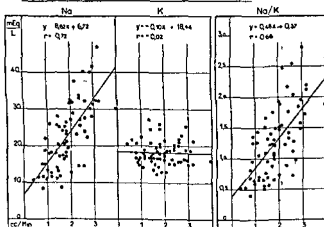


FIG. 1.

analysed in the same way. The values obtained from male and female adults and children show no significant difference when the volume is taken into account.

Method of Stimulation. In our group of women we have studied unstimulated and paraffin-stimulated saliva. We have compared these results with those of Warming-Lârsen *et al.* (1952) who used pilocarpine as a stimulant. The flow, Na and Na/K results are lowest for unstimulated saliva, intermediate for paraffin-stimulated and highest for pilocarpine-stimulated saliva, while the K results show no significant

THE Na AND K CONCENTRATION IN MIXED SALIVA: INFLUENCE OF SECRETION RATE, STIMULATION, METHOD OF COLLECTION, AGE, SEX, TIME OF DAY AND ADRENOCORTICAL ACTIVITY

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ACCORDING to Frawley and Thorn (1951) the Na/K ratio of saliva is raised in adrenal insufficiency (Addison's disease) and lowered in adrenal hyperfunction (Cushing's syndrome). Despite its simplicity the saliva test has not been generally adopted for the diagnosis of adrenal abnormality, the principal reasons being that different authors give different normal values and that the factors influencing the saliva electrolyte concentrations are not fully understood.

The first part of this paper describes the normal Na and K concentrations and how they are influenced by collection and stimulation methods, rate of flow, sex, age and time of day. Furthermore we have studied whether or not one individual's results remain constant over a period of time. The second section deals with the effect of adrenocortical disorders, stress, ACTH and adrenocortical hormones.

Unless otherwise stated the saliva was collected before 8 a.m. Paraffin was chewed for two consecutive five-minute periods without swallowing any saliva and each portion of saliva was collected in a separate container. Only the second portion was analysed. A Beckman flame photometer was used to determine the Na and K content.

Rate of Flow. Gregersen and Ingalls (1931), Baxter (1933) and Brown and Klotz (1937) have already observed that the

the slope of the regression line (Table I). The permissible deviation at 2 ml./min. is then taken as the normal range for corrected Na and Na/K (Table I).

Table I

CORRECTION OF Na, K AND Na/K VALUES IN SALIVA TO A STANDARD FLOW OF 2 ML./MIN. (10 ML./5 MIN.).
(saliva collected during five minutes, Na and K in mEq/l, volume in ml.)

Na_c	=	$Na + (10 - vol./5\ min.) \times 1{\cdot}6$
K_c	=	K
$(Na/K)_c$	=	$Na/K + (10 - vol./5\ min.) \times 0{\cdot}1$

NORMAL Na, K AND Na/K VALUES IN SALIVA (MEAN $\pm 2\sigma$)
(second five-minute period in the morning with or without stimulation, corrected (c) to a flow of 2 ml./min. = 10 ml./5 min.)

Na_c	=	12	—	36 mEq/l
K	=	11	—	27 mEq/l
$(Na/K)_c$	=	0 6	—	2 1

Method of Collection. The above values were obtained from the saliva of the second five-minute period. When three adults chewed paraffin for twenty minutes the Na concentration of their saliva, using the second five-minute period as a standard, was 13 per cent higher in the first five minutes and 5 per cent lower thereafter. The K concentration stayed practically the same.

Newborns. In infants we aspirated the saliva for a period of ten minutes. It is unlikely that the very small volume obtained represents the true saliva volume. It is therefore impossible to correct for flow. The results obtained from infants over three months of age (Fig. 3) are within the normal variation of unstimulated saliva values of children and adults as shown in Fig. 2. On the other hand the Na and Na/K values in newborns are considerably higher and reach normal values only after several weeks (Fig. 3). This would suggest a transitory physiological adrenocortical insufficiency.

change. The regression lines for all three groups are virtually the same; that is, the effect of flow on Na and Na/K is the same regardless of the method of stimulation. When this effect of flow is taken into account, the values for unstimulated and stimulated saliva show no significant difference.

Altogether we have analysed 150 specimens of early morning saliva, both unstimulated and paraffin-stimulated, obtained

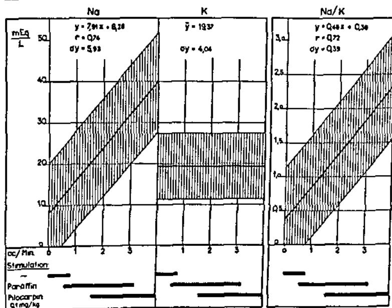


FIG. 2.

from healthy adults and children. Fig. 2 gives the mean and the permissible deviation (2σ) as well as the areas covered by unstimulated, paraffin-stimulated and pilocarpine-stimulated saliva. As in Fig. 1 the Na concentration and the Na/K ratio vary with flow, while the K concentration is independent. This graph shows clearly that Na and Na/K values of unknown flow have at best a relative meaning. In order to compare individual Na and Na/K values we have corrected them to a standard volume of 2 ml./min. using for this correction

hildren we studied their saliva values throughout the day Fig. 4). The result, confirming that of Grad (1951), shows hat the Na concentration is highest in the morning. Ambula-

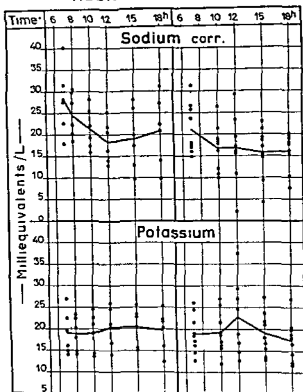


FIG. 4.

tory and bedridden patients show the same diurnal variation of the saliva electrolytes, whereas the diurnal variation of the urine electrolytes is known to vary with body position.

Repeated Determinations on the Same Individual. *Saliva was obtained from two healthy adults throughout the*

Time of Day. In our group of women we found significantly higher Na values upon arising than two hours later.

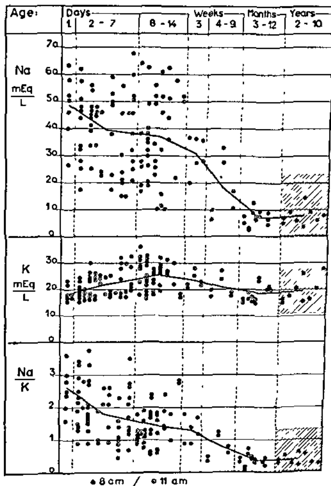


FIG. 3.

In the group of children the same phenomenon appeared although the difference was not significant. The K concentration remained constant. Taking a sample of adults and

children we studied their saliva values throughout the day (Fig. 4). The result, confirming that of Grad (1951), shows that the Na concentration is highest in the morning. Ambula-

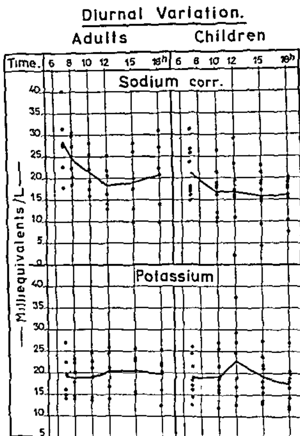


FIG. 4.

tory and bedridden patients show the same diurnal variation of the saliva electrolytes, whereas the diurnal variation of the urine electrolytes is known to vary with body position.

Repeated Determinations on the Same Individual. Saliva was obtained from two healthy adults throughout the

course of one day and this was repeated on three occasions at monthly intervals (Fig. 5). The values for each individual remained remarkably constant, even though the values of

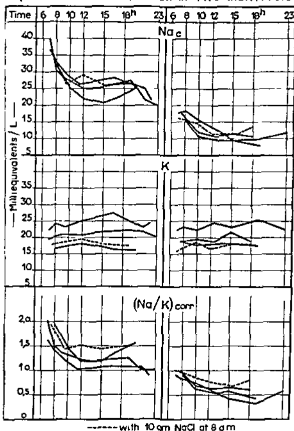


Fig. 5.

one were in the upper normal range and the values of the other in the lower normal range.

Adrenocortical Disorders. Figure 6 shows our findings in patients with hyper- and hypoadrenocortical states. The three cases of *Cushing's syndrome*, all showing markedly

creased urine and plasma corticoids, have, contrary to what e expected, normal Na_c and $(Na/K)_c$ values, although two ave raised K values. In the *congenital adrenogenital syndrome* e found normal values. The case of Cushing's syndrome and

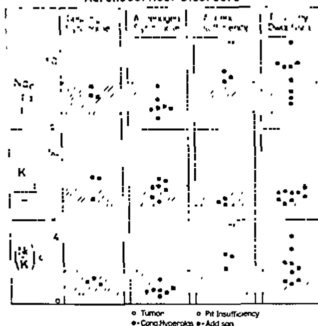


Fig. 6.

the case of the adrenogenital syndrome caused by an *adrenal tumour* showed virtually the same values before and twelve days after the removal of the tumour. The three patients with untreated *Addison's disease*, but normal serum electrolytes, have definitely increased Na_c and $(Na/K)_c$ values and rather low K values. This confirms the findings of Frawley and Thorn (1951). An extremely high Na_c value was obtained from a case of *pituitary insufficiency* caused by a tumour

metastasis in the pituitary stalk. In contrast to the resul' obtained from pituitary and adrenal insufficiency, five cases (*anorexia nervosa* showed normal saliva values. Results simil to those of Addison's disease were found in several cases (*pituitary dwarfism*. Even though the serum electrolytes wei normal and there was no manifest adrenal insufficiency these saliva results point to an insufficient productio of ACTH. Urine electrolyte studies carried out unde normal and lowered NaCl intake in one case of pituitar dwarfism showed an insufficient renal retention of NaCl a found in adrenal insufficiency. On the basis of these result we are now regularly using the saliva electrolyte test i addition to the insulin tolerance test and the water excretion test as a diagnostic aid for pituitary dwarfism. In our ex perience, however, not all three tests must be abnormal As Luetscher and Johnson (1954) found an increased excre tion of salt-retaining steroids in children with *nephroti syndrome*, we expected to find a decreased $(Na/K)_c$ ratio ii saliva. However, we obtained normal values in the fou severe nephrotics that we examined.

Considered as a whole, these results show that in hyper adrenocorticism the saliva electrolyte test is of uncertain value, while in hypoadrenocorticism it has a definite diagno stic value.

The Effect of Stress and of an Eight-Hour Intravenous Infusion of ACTH. We studied the saliva of six children before, and eight and twenty-four hours after an operation under general anæsthesia. After eight hours, despite a 99 per cent fall in eosinophils, the $(Na/K)_c$ ratio did not drop as markedly as in the controls (Table II). After twenty-four hours, the saliva values obtained were essentially the same as before the operation. After an eight-hour intravenous ACTH infusion carried out on five cases, the saliva electrolytes were within the normal diurnal variation (Table II). Both tests show that the $(Na/K)_c$ ratio does not decrease after intensive stress or after an eight-hour i.v. ACTH infusion. This is in contrast to the fall in eosinophils and to

Table II

SALIVA AND EOSINOPHIL PERCENTILE CHANGE AT 4 P.M. COMPARED TO 8 A.M.

	Number of Cases	Na_c	K	$(Na/K)_c$	Eosinophils
1. Adults . .	6	−21	+ 9	−30	
2. Children . .	8	−17	0	−15	
3. Children, operated at 8 a.m.	6	+ 5	+15	− 1	−99
4. Children and *adolescents*, ACTH (12 mg./m²) i.v. from 8a m. to 4p m	5	−18	+10	−19	−96

the marked change in urine electrolytes (Thorn *et al.*, 1953; Holland and Stead, 1954).

significant. This result is in contrast to our surgical stress experiment where a much better response in the eosinophils but *no response in the* saliva electrolytes was obtained. It seems that the saliva electrolytes react differently to different forms of stress and that the electrolyte effect is independent of the eosinopenic effect.

The Effect of Prolonged Cortisone and ACTH Therapy. Four hours after 100 mg. of cortisone p.o., two healthy adults showed an average eosinophil drop of 69 per cent but no change in saliva electrolytes. After 250 mg. cortisone p.o. in one twenty-four-hour period the same subjects showed an average eosinophil drop of 58 per cent and a $(Na/K)_c$ drop of 23 per cent. Twenty-four hours later the eosinophils were still 17 per cent and the $(Na/K)_c$ ratio 11 per cent lower than at the beginning. After four i.v. injections of 20 mg. ACTH

given during one twenty-four-hour period, two healthy adults showed an eosinophil fall of 89 per cent and a fall of the $(Na/K)_c$ ratio of 30 per cent. In 13 patients with leukæmia, rheumatic carditis and the nephrotic syndrome, treated for more than three days with cortisone or ACTH, the average fall of Na_c was 16 per cent, the rise of K 12 per cent and the fall of $(Na/K)_c$ 19 per cent. Eleven of the thirteen patients had a lower $(Na/K)_c$ ratio than before the therapy. These results confirm those of Frawley and Thorn (1951), Dreizen *et al.* (1952), Warming-Larsen *et al.* (1952) and Grad (1952).

The Effect of Desoxycorticosterone (DOC). Three healthy adults, two and four hours after one i.v. injection of 50 mg. DOC-glycoside and twenty-four and forty-eight hours after one i.m. injection of 15 mg. DOC-acetate showed no definite change in saliva values. In another case where 500 mg. DOC-acetate was implanted as pellets, the $(Na/K)_c$ ratio decreased 1 per cent in twenty-four hours, 16 per cent in forty-eight hours and 27 per cent in seventy-two hours.

The Effect of an Increased Intake of NaCl. After 10 g. of NaCl taken at 8 a.m. by two adults the decrease of the $(Na/K)_c$ ratio during the day was less marked than during the control periods in the same two individuals. (Fig. 5).

Summary

(1) The Na concentration and the Na/K ratio of normal mixed saliva depend on secretion rate, duration of stimulation and the time of day, whereas the K concentration is practically independent.

(2) One can only obtain comparable results when the time of day and the duration of stimulation are kept constant and when one corrects for the secretion rate. When saliva is collected in the morning for two consecutive five-minute periods and when the values of the second five-minute period are corrected to a flow of 2 ml./min. one obtains the following normal values (mean $\pm 2\sigma$):

Na 12–36 mEq./l., K 11–27 mEq./l., Na/K 0·6–2·1

(3) Under these conditions unstimulated, paraffin-stimulated and pilocarpine-stimulated saliva give the same results.

(4) There is no significant difference between the values for adult males, adult females and children, whereas in the saliva of newborns the Na and Na/K values are increased, suggesting a transitory adrenocortical insufficiency.

(5) In the same individual the saliva electrolyte values remain remarkably constant.

(6) In adrenocortical insufficiency with normal serum electrolytes the Na/K ratio of saliva is increased. This finding is of considerable diagnostic aid, especially for the problem of pituitary dwarfism in children.

(7) In the adrenogenital syndrome the Na/K ratio is normal. Contrary to others we found a normal ratio in three cases of Cushing's syndrome.

(8) In the nephrotic syndrome, where there is a known increased excretion of salt-retaining steroids, the Na/K ratio of saliva is normal.

(9) An increased intake of NaCl increases the Na/K ratio.

(10) ACTH, cortisone and DOC lower the Na/K ratio, but the effect on the saliva electrolytes is less marked and appears to be slower than the effect on the eosinophils and on the urine electrolytes.

(11) Eight hours after a surgical stress with an eosinophil drop of 99 per cent the Na/K ratio does not show any decrease.

Acknowledgements

We wish to express our thanks to Dr. G. Forster and Dr. A. Labhart as well as to the staff of the Dept. of Internal Medicine of the University Hospital in Zurich who obtained saliva samples for us and to Miss R. Inbelder, Miss S. Stahel, Mr. F. Hermann and Mr. W. Adank who gave us technical assistance.

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DISCUSSION

WILKINS: Were your cases of adrenogenital syndrome of the losing type?

PRADER: No. The ones I have shown are only older cases wi salt-losing syndrome. In t
found normal values for t
intake of sodium chloride,
chloride, and lower values if we added ACTH.

BROWNE: In connection with the effect of operation, did you no a decrease at any time after operation?

PRADER No, but we collected saliva only eight and twenty-four after operation.

THORN Were these surgical patients receiving an intrave infusion of sodium chloride as post-operative therapy?

PRADER: No

ELMADJIAN: In Korea we found that in individuals who sh

urine were very sharp in their response.

THORN. The lack of correlation with stress is obvious, but the t that is interesting is the special salt-retaining hormone effect. massive doses of cortisone one can get marked salt retention by kidney but comparatively very little effect on the salivary ratio. Mach, was there any effect on salivary ratio in your patients g aldosterone?

MACH: We checked the salivary ratio in some medical students.

than by biological assay.

BROWNE: I gather that Dr. Prader did correct the flow in connection with the stress response, because one does get quite a marked decrease in flow.

PRADER: Yes, the values are corrected for flow. However, we did not observe a decrease in flow, probably because of the paraffin stimulation.

BROWNE: In a case of Cushing's syndrome which I'm showing tomorrow there was a marked electrolyte disturbance in the blood and a marked decrease in salivary sodium/potassium ratio. After a 9/10 adrenalectomy on the left side and a total right adrenalectomy on November 23, 1953, it rose, and by January 31, 1954 had fallen again. Regeneration of the remnant to 16 g. weight was found to have occurred when he died in April, 1954.

PRADER: I may add that one of these cases of Cushing's syndrome was operated on because of an adrenal tumour, and that after the operation we found nearly the same saliva values as before, although the urine and plasma corticoids had dropped to a very low level.

QUERIDO: I wonder whether the textbook story that the normal person cannot be made hypertensive with high doses of DCA is true. I have no experience of that.

GROSS: It depends on the sodium content.

hypertension which proved completely reversible by stopping the DCA for three to four weeks.

QUERIDO. So a normal person could be sensitive?

FORSHAM If you call a person with psoriasis normal, apparently yes.

MACH: We gave 100 mg. DCA during five days, and we observed a small effect on retention of sodium and water, but no effect on the blood pressure.

are such marked physiological changes in the kidney and everywhere else, I think that, in the absence of other proof, we should not accept the changes in salivary ratio as necessarily being dependent on changes in adrenal function.

AN ATTEMPT TO CORRELATE THE HISTOLOGICAL CHANGES IN THE ANTERIOR HYPOPHYSIS AND ADRENAL GLANDS IN VARIOUS DISEASES IN MAN

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IN the first part of this symposium, Symington described the histological changes in the adrenal glands in a variety of diseases. This communication is a corollary and embodies an attempt to correlate the pituitary and adrenal changes in 96 cases.

So far as we are aware there is no other record of any combined cytological study of the pituitary and adrenal in conditions of stress in man, though several descriptions have been published of the histological appearance of the adrenal cortex (Ayres *et al.*, 1951; Stoner *et al.*, 1953; Sarason, 1943; Zamcheck, 1947; Rogers and Williams, 1948, and Symington *et al.*, 1954).

The evidence of the exact source of ACTH in man is of an indirect nature and it is only recently that it has been prepared from human pituitaries (Hewett, Cruickshank and Currie, 1954). Marshall (1951) demonstrated conclusively that it is produced by the basophils of the anterior hypophysis in the hog. The changes which occur in the basophil cells in Cushing's syndrome (Crooke, 1935), in Addison's disease (Crooke and Russell, 1935) and as a result of cortisone administration (Laqueur, 1950, 1951; Bennett, 1953) support the view that in man also ACTH is produced and secreted by them. Accordingly, it seemed reasonable to study the basophils in stress conditions with a view to correlating, if possible, their appearance with the various adrenal patterns which

:ur and which have been described in detail by Symington, rrie, Curran and Davidson (1954).

Material

Autopsy examinations were performed within a few hours death. The patients died as a result of a variety of diseases :luding *hypertension, coronary artery disease, carcinomas* bowel, stomach and lung, acute infections such as pneuonia, burns, after operations on acute suppurative appendi:is and perforated peptic ulcers with peritonitis, and after :ctive surgery. In our experience, pituitary and adrenal aterial obtained more than eight hours after death is isatisfactory for detailed microscopic examination.

Methods

Adrenal. Thin slices of the adrenals were fixed in 10 per :nt neutral formol-calcium solution, embedded in paraffin id in gelatine. Gelatine-embedded material was cut at 11μ id the lipids stained by Hæmatoxylin and Sudan IV and udan Black. The methods used for the demonstration of *iolesterol, the "ketosteroid" reactions, ribonucleic acid* RNA) and deoxyribonucleic acid (DNA), alkaline phosphatase nd non-specific esterase have been described previously Symington *et al.*, 1954).

Anterior Hypophysis. Immediately after removal the ituitaries were halved horizontally with a razor blade and arious fixatives were used. We have obtained the best esults with 10 per cent neutral formol-calcium and formol orrosive fixation and we are in full agreement with Pearse 1952) that Bouin's fluid is unsatisfactory for the method of xamination used for this investigation. Paraffin sections of μ were stained by the trichrome periodic-acid Schiff echnique (P.A.S.).

Assessment of Adrenal and Pituitary Changes

Assessment of Adrenal Patterns. Most of the previous eports on adrenal patterns (Ayres *et al.*; Stoner *et al.*; Sarason;

Zamcheck; Rogers and Williams) are based solely on the variable distribution and size of the adrenal lipid globules, but in a recent investigation (Symington *et al.*) the significance of factors such as alteration in cell type, presence of degenerative changes in the cortical cells, distribution of RNA and cytoplasmic enzymes (alkaline and acid phosphatase, and non-specific esterase) have been considered in conjunction with the distribution and character of the adrenal lipids. Four main patterns are found and they occur with minor variations in the child as well as in the adult gland:—

1. "Normal" lipid-laden adrenal. (Fig. 1).
2. Focal lipid depletion (Fig. 2).
3. Extreme diffuse lipid depletion.
4. Lipid reversion (a type of focal depletion). (Fig. 3).

For the purpose of this investigation the glands have been assessed from O to ++++ depending mainly on the degree of lipid depletion and to a lesser extent on the cytology and the distribution of RNA and enzymes (Table I). These

Table I

ASSESSMENT OF ADRENAL PATTERNS

Adrenal Pattern	*Assessment*
[illegible]	O
[illegible]	+
[illegible]	++
[illegible]	+++
[illegible]	++++

assessments are based on the histology of the adrenal cortex and we wish to emphasise that they do not necessarily represent degrees of secretory activity of the gland. Each case was given its assessment without knowledge of the pituitary results.

Assessment of Pituitary Changes. The problem of the assessment of basophil changes had been in our minds for some time when Pearse's papers were published (1952)

Fig 1. *"Normal" Lipid-laden Adrenal* All zones contain abundant lipid. The thin ill-defined zona glomerulosa is seen above. Hæmalum and Sudan IV. ×25.

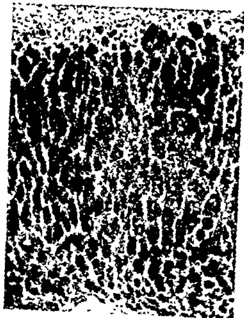




Fig. 2 *Focal Lipid Depletion*. Some areas of the cortex contain abundant birefringent lipid and



FIG 3 *Lipid Reversion* Abundant lipid is present in the inner part of the cortex Hæmalum and Sudan IV. ×45

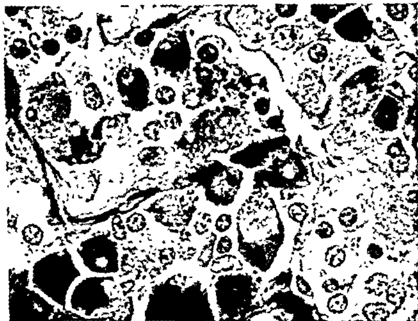


Fig 4 *Mucoid Cells* Almost all the cells in this field are mucoid cells Note the difference in the degree of granulation The maximal cells are heavily granulated while the intermediate cells are not Trichrome periodic-acid Schiff ×850

should be classified as a maximal or intermediate and an Ilford Spectrum green filter (5000—5400Å) was helpful and was used throughout for this purpose (Pearse).

We do not claim that this method gives an accurate result but with experience and using suitably-fixed, fresh material

As in the case of the adrenal the glands are classified from 0 to ++++ for the purpose of correlation (Table II). The

Table II

Assessment of Pituitary Changes

M/I Ratio	*Assessment*	
More than 1/2	O	
1/2–1/2·5	+	"Normal" according to Pearse (1952)
1/2·5–1/3	++	
1/3–1/3·5	+++	
Less than 1/3·5	++++	

assignation of O to ++++ depends on the M/I ratio—on the degree of degranulation of the mucoid cells—and once again we would emphasise that these assessments do not necessarily indicate varying degrees of secretory activity. The counts were done without knowledge of the adrenal assessments and in most cases without knowledge of the cause of death.

Results

The detailed results of the pituitary and adrenal findings in each case are not within the scope of this paper. In general, in most cases dying as a result of acute infections, burns, post-operatively and of hæmorrhage, the M/I ratio is less than "normal" (++ – ++++), while in those dying of more chronic illnesses, such as hypertension and malignant disease without superadded complications, the M/I ratio is "normal" (+) or greater than normal(0). The main adrenal findings

have been given previously (Symington *et al.*). Fig. 6 contains the results of 96 cases assessed by the methods we have described.

There are 56 cases in the + and ++ adrenal groups, i.e., 28 cases showing 1–25 per cent and 28 with 25–50 per cent lipid depletion of the cortex, and the pituitary assessments

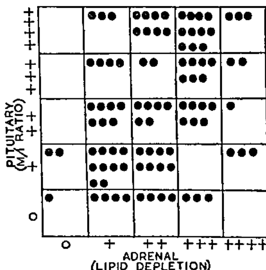


FIG. 6 M/I ratios (degrees of degranulation) of mucoid cells of the anterior hypophysis and degrees of lipid depletion of adrenal glands in 96 cases.

present a wide scatter. The adrenals of 30 cases out of 56 are associated with pituitaries with an M/I ratio of *less* than "normal" (++ – ++++). There are only three cases with completely lipid-loaded adrenals (0) and in our experience (see also Symington *et al.*) this is not a common picture. It would appear that the "normal" adrenal cortex of non-stress states is one showing approximately 1–5 per cent lipid depletion and it therefore falls into the + group. This might explain the presence in the + adrenal group of 14 out of 28

pituitaries with a + or 0 assessment, but it does not account for almost half the cases (12 out of 28) in this category in the ++ adrenal group (25–50 per cent lipid depletion).

With the exception of three cases, two of which had large space-taking lesions of the pituitary, the pituitaries of the +++ adrenal group (lipid depletion of 50–95 per cent) show M/I ratios of less than "normal", 11 of a total of 28 having a ++++ pituitary assessment.

In the ++++ adrenal group there are nine cases and the pituitaries again present variable findings. Three of them have "normal" M/I ratios (+) and three very low M/I ratios (++++).

From these results it is apparent that adrenals with all degrees of lipid depletion may be associated with pituitaries presenting a wide range of results in terms of the M/I ratio. In an attempt to clarify these apparent anomalies an analysis of the post-mortem records and clinical histories of the cases was undertaken to separate those in which it was possible to time the onset of an acute stress. It is often difficult in human beings to time accurately the onset of stress and to estimate its severity and duration, but fortunately in 44 out of the 96 cases the illness or trauma was of acute onset. This group includes patients who died from burns of more than 20 per cent of the skin surface, from coronary thrombosis, lobar pneumonia and other acute infections, perforation of peptic ulcers, acute suppurative appendicitis and after elective surgery. These cases are comparable only with regard to their acute onset; it is not claimed that there is any uniformity in the degree of stress. Naturally, there was a great variation in the interval between the onset of the stress and death. The results of those cases are shown in Fig. 7.

There is a closer correlation between the pituitary and adrenal assessments and there are fewer apparent anomalies. Most of the lipid-depleted adrenals are associated with pituitaries presenting M/I ratios of less than normal (++ – ++++). In the + adrenal group seven out of eight cases show a pituitary assessment of ++ – ++++. In the ++

and +++ adrenal groups only 3 out of 28 cases have pituitaries with M/I ratios of "normal" (+) or more than normal (O). The same scatter, however, is still present in the ++++ adrenal group.

If the pituitary assessments are charted against the time in weeks between the onset of the stress and the death of the

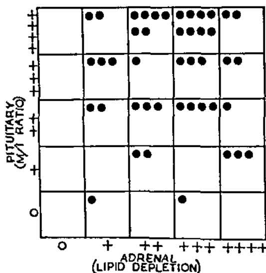


FIG. 7. M/I ratios of mucoid cells of the anterior hypophysis and lipid depletion of adrenal glands in 44 cases in which illness or trauma was of acute onset.

patient an interesting picture is presented (Fig. 8). In the first week 22 out of 23 cases show decreased M/I ratios and 14 of these are in the ++++ group. This marked degree of degranulation has been seen in four cases out of a possible five in the first thirty-six hours. There is a reasonable explanation for the case with an "0" assessment during this period. The patient died within eighteen hours of a hysterectomy for myomata of the uterus. She rallied from the anæsthetic but

died shortly afterwards in her sleep. After the first week there is a definite tendency for the M/I ratio to increase and by the fifth to sixth week the three cases in this group present a "normal" result (+). These three were all burns cases and from the findings in six other patients who died of burns during the first week it may reasonably be assumed that their

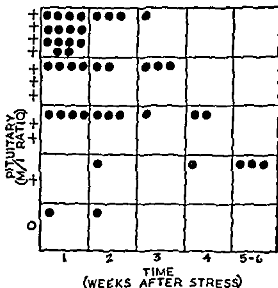


FIG. 8. M/I ratios of mucoid cells of the anterior hypophysis and time in weeks from onset of acute stress to the death of the patient in 44 cases.

M/I ratios at that time were greatly decreased (+++ - ++++).

Now if the adrenal assessments are charted against time in the same way (Fig. 9) all the glands show evidence of lipid depletion in the first week and most of them are awarded ++ and +++ assessments. It is of interest that the ++++ lipid-depleted gland in this group was found in a child of 12 who died four days after a burn which involved 25 per ent of the body surface. The earliest ++ depletion was seen fter twenty-one hours and most of the + depleted glands

were found in patients who died within one day of the onset of the stress. Seven of the glands present ++++ depletion after the first week and this contrasts with the findings in the pituitary (Fig. 8). Ten of the 14 with + – +++ depletion between the beginning of the second week and the end of the sixth present the reversion pattern of Sarason while only 7

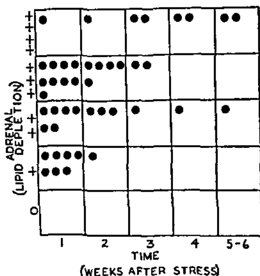


FIG. 9. Adrenal lipid depletion and time in weeks from onset of acute stress to the death of the patient in 44 cases.

of the 23 cases dying within the first seven days show this lipid distribution, which is seldom seen before the fourth day. The three cases who died in the fifth to sixth week are of particular interest. They were all associated with "normal" (+) pituitaries and the one with a ++ assessment showed the reversion pattern.

Discussion

We are fully aware of the dangers of reading too much into static histological pictures and of translating them into terms

of activity. So far we have presented our results in the form of degrees of degranulation of the mucoid cells, i.e., by the M/I ratios, and of lipid depletion of the adrenal cortex. Pearse has suggested that the M/I ratio can be used as an index of the secretory activity of the mucoid cells. By this he assumes that all the degranulate intermediate cells of all types have unloaded and are unloading mucoprotein, but the mucoid cells must surely pass through the intermediate stage in the process of stocking mucoprotein and becoming maximal in type. The vesiculate cells are included in the intermediate count and Pearse believes that they may be used as an index of activity of production of mucoprotein. In some glands they account for more than 15 per cent of the mucoid cells and it is not known whether the vesiculate cells and other degranulate forms can secrete, produce and store simultaneously. The findings presented in Fig. 8 suggest that reversion of the mucoid cells may occur before death.

In the same way it has been accepted by some workers in the past that lipid depletion of the adrenal of any degree up to, but not including, the completely lipid-depleted cortex indicates an actively-secreting gland. If, however, a lipid-depleted gland is in the process of reversion to the "normal" pattern it must restock with lipid and death may intervene before the process is complete. We believe that this may be represented by the reversion pattern of Sarason (Fig. 3). It is probable that the presence of alkaline phosphatase and RNA in the compact cells signifies active production but not necessarily secretory activity.

Consequently, if we accept the hypothesis that reversion of the pituitary and adrenal to normal may begin before the death of the patient it is clear that it is very dangerous to interpret in all cases that the assessments based on the M/I ratio and the lipid-enzyme-RNA distribution in the adrenal are synonymous with the activity of secretion of the mucoid cells of the anterior hypophysis and the adrenal cortex respectively. They may certainly be actively producing their respective hormones but it is unlikely that glands which

are reverting to "normal" are secreting more than the "normal" requirements.

In the first week or so after an acute stress, however, it seems reasonable to accept degranulation of the mucoid cells of the anterior hypophysis and adrenal lipid depletion as the structural changes, associated with eosinophil counts of 0 and increased urinary acid-stable formaldehydogenic steroids (F.S.S.) (Symington *et al.*). During this period we believe that the decrease in the M/I ratio (++ – ++++) reflects fairly accurately the activity of secretion of the mucoid cells and presumably of adrenocorticotrophic hormone (ACTH). In some cases there is pronounced P.A.S.-positive plasma (Fig. 5) and occasionally P.A S.-positive granules in the pituitary capillaries. These findings have been most prominent in cases with decreased M/I ratios (+++ and ++++) but are not constantly present in them. Similarly, the lipid depletion occurring as a result of stress is presumably due to the out-pouring of adrenocortical hormone, although we would emphsasise that we are aware that we do not demonstrate cortical hormone or ACTH histochemically.

If, at this stage, we reconsider the results shown in Fig. 6, reasonable explanations for the apparent anomalies are readily available and may account for some of them. Included in the + adrenal group (1–25 per cent lipid depletion) are cases of the "steady flow" associated with pituitaries presenting an 0 – + assessment. In addition, there are some cases dying soon after the onset of the stress and in these the pituitaries show a very pronounced degree of degranulation (+++ and ++++) and this change precedes the lipid depletion of the adrenal (Figs. 8 and 9). The + lipid-depleted adrenal, therefore, may represent a gland which is secreting "normally" or it may represent one which is extremely active or, indeed, any other grade of secretory activity between these.

In the ++++ adrenal group, the so-called "exhausted" adrenal with complete or almost complete depletion of lipid, only one of them occurred within the first seven days (Fig. 9)

and it was associated with a pituitary with a markedly decreased M/I ratio (++++). In the fifth to sixth weeks there are two glands showing complete depletion and the M/I ratios in these were assessed at +, the "normal" of Pearse. Cortical hormone may depress ACTH secretion and it seems reasonable to postulate that this is the mechanism responsible for these apparent anomalies. If this is so these ++++ depleted adrenals are active and not "exhausted" glands. Some of the + and 0 pituitaries associated with the + and ++ lipid-depleted adrenals (Fig. 6) may perhaps be accounted for in a similar fashion. To lend further support to this argument we would add that one of the cases in this category received large doses of cortisone for several days before death and it seems logical that the pituitary should revert to a normal pattern before the adrenal. We must not forget, however, that ACTH is the main but not the sole controller of the adrenal cortex. Clearly the varying degrees of lipid depletion in the adrenal may represent any degree of secretory activity and, in our view, the extent of the depletion is more likely to be indicative of the severity and duration of the stress.

We submit that the changes shown in the mucoid cells in response to stress lend further support to the view that ACTH is formed and secreted by them, as they occur along with lipid depletion of the adrenal cortex, low eosinophil counts and an increase in the urinary acid-stable F.S.S. The mucoid cells, however, are also believed to be the site of formation of thyrotrophic hormone (TSH.), luteinising hormone and follicle-stimulating hormone, but neither these nor ACTH is detectable histochemically. It is not known whether all the mucoid cells are responsible for the secretion of all these hormones or whether groups of cells elaborate one only. It may be argued, therefore, that similar structural changes may occur in these cells as a result of secretion of any one of these hormones. In this connection it is of interest that the highest vesiculate cell count we have seen was in a case of myxœdema where it is likely that it indicated active production of TSH and not of ACTH. Going on the assumption

that all these hormones are produced and secreted by the same cell, the work of Harris (1954) is of particular interest. From his results it would appear that in response to stress the mucoid cells secreted ACTH at the expense of TSH.

In the adrenal cortex the sugar-active and androgenic

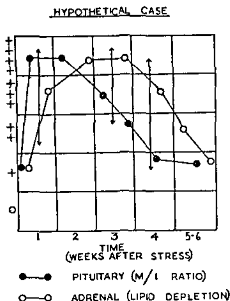


FIG. 10 *Hypothetical Case.* Chart of possible M/I ratios and degrees of adrenal lipid depletion from onset of acute stress. *If the patient dies in the first week (arrow) the pituitary may* show a ++++ assessment and the adrenal ++ lipid depletion In the third week (arrow) the lipid depletion is pronounced (++++) while the degree of granulation of the mucoid cells is greater (+++) In the fourth week an adrenal showing +++ lipid depletion may be associated with a pituitary presenting either ++ or + ("normal") assessment.

hormones are produced presumably by the same cells. The compact cell is seen in response to stress, in Cushing's syndrome and in the adrenogenital syndrome (Symington *et al.*).

The results may perhaps be best summarised by charting the M/I ratio and adrenal lipid depletion in a hypothetical case (Fig. 10). If we assume that death may occur at any

stage from the onset of the stress to the end of the sixth week a great variety of results may be obtained from one day to another or from one week to another. In the first week a pituitary assessed at ++++ may be associated with an adrenal showing + – +++ lipid depletion; in the second week a ++++ pituitary may be found with a +++ or ++++ adrenal or the latter may be seen with a +++ pituitary. About this time the pituitary is beginning to revert to normal; active production continues but secretion is probably reduced to satisfy "normal" requirements. In the third week a ++++ lipid-depleted adrenal is associated with a +++ or ++ pituitary and in the fourth week the adrenal begins to revert to normal. The degree of histological change produced and the time taken for the glands to recover must vary not only with the severity and duration of the stress but also with the response of the individual.

Clearly the degree of lipid-depletion and the degree of degranulation of the mucoid cells are not necessarily synonymous with the secretory activity of the adrenal cortex on the one hand or with that of the mucoid cells of the anterior hypophysis on the other, e.g., a + lipid-depleted adrenal may be secreting cortical hormone very actively, whereas one presenting +++ lipid depletion may be in reversion and secreting only "normal" requirements. The histological findings must be correlated with the clinical pictures and with the biological assays available. The supply, demand and utilization of ACTH and adrenocortical hormone are difficult factors to estimate but we believe that the advent of satisfactory methods for urinary and blood corticoids and for urinary and blood ACTH levels, combined with the methods which we have employed, should add greatly to our knowledge of the physiology and pathology of the pituitary and adrenal glands in conditions of stress.

Acknowledgements

We thank with pleasure Professor G. L. Montgomery for his interest, advice and encouragement; Mr. P. J. Elliot for the photography and Mr. Donald C. F. Hay for technical assistance.

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DISCUSSION

pituitary

CURRIE: Most of our cases show lipid-depletion of the adrenal cortex

CURRIE: Yes, I think that it is more or less accepted now that Crooke's hyaline cells are secondary to hyperfunction of the adrenal cortex.

BROWNE: Have Crooke cells been found in people treated with high doses of cortisone that have died?

CURRIE: Hyaline changes in the basophils have been described in patients treated with cortisone.

BROWNE: After how long a period of treatment with cortisone?

CURRIE: Laqueur, for example, claims that changes may occur very quickly—within a matter of days.

ZUCKERMAN. In the first half of this colloquium, Professor Yoffey, who gave a paper describing various histological changes in the adrenal,

asked whether these could be correlated with the functional state of the adrenal cortex. I seem to recall that Dr. Pincus remarked that the adrenal glands which are perfused *in vitro* in his laboratory are completely depleted of any of these materials which show up histologically,

from a variety of diseases and discussed the functional significance of the various patterns. It is dangerous to deduce too much from a static histological picture alone. It must be correlated with the clinical condition of the patient and with the various tests for adrenocortical activity—such as eosinophil counts and urinary steroids. Lipid depletion of the cortex in most cases probably signifies an actively secreting gland but it does not necessarily follow that the degree of lipid-depletion parallels the activity of secretion of adrenocortical hormone. I hoped to make it clear that a + lipid-depleted adrenal cortex may represent a
lipid-depleted cortex in
ments only. In the sam

picture throughout the adrenal cortex?

CURRIE. In the "normal" lipid-laden adrenal the whole of the cortex contains lipid. In focal depletion there is a tremendous range, as we have shown, in the degree of lipid depletion In extreme diffuse depletion the whole cortex is lipid-depleted.

not gone into this problem in detail The ages, the weights, the heights of the patients differ and they must be taken into account.

VOGT: In the experimental animal the adrenals would be enlarged and I wondered whether in man depletion could occur so rapidly that there would be no time for hypertrophy, so to speak?

CURRIE: We have seen an adrenal showing almost complete depletion in four days.

VOGT: In an animal four days would be enough to produce hyper-

by Dr Vogt. Under
. II or chronic exposure
to stress we observed in the first few hours a maximal depletion of the adrenal lipids in the rat. This was followed by a gradual repletion, which could be correlated with the degree of hypertrophy (Fortier *et al.*, 1950, *Endocrinology*, 46, 21). The explanation has been suggested by Sayers (*Physiol. Rev.*, 1950, 30, 241) that with the appearance of a greater number of functional units the adrenal function could be re-established at a new steady state.

CURRIE. It is not easy to control conditions in man—so many complications may arise. It is difficult to know how many of the experimental findings can be applied to man. There is no doubt that the manner in which the adrenal cortex of the experimental animal depletes is different from man.

PRUNTY: I don't know whether this group would accept the idea that the rate of depletion of lipid material in the cortex has some correlation

[illegible]

temporal relationship between hormone discharge and lipoid depletion and repletion is clear-cut, whereas in man you've got a complicated situation, a burn is sustained and then all sorts of factors come in, treatment is applied and so on. The multiple stimuli set up tend to put this uniform sequence of events out of phase.

CURRIE: Yes, I do agree that in man it is complicated. We did stress this in the first part of the symposium. The dressing of severe burns may result in increased excretion of acid-stable F.S.S in the urine.

COPE. It seems probable on general grounds that the storage that *you see is a balance between the depletion and the storing up again*, doesn't it? One of the factors that may well have an influence on this balance is what one might loosely call the toxæmic state of the patient. Certainly in some very severe fatal septicæmias you find depletion of the adrenal gland. I wondered whether you had noticed any difference in your histological pattern of the adrenal between the extreme types where there was no evidence of septicæmia (possibly a coronary thrombosis or a post-operative sudden embolus) on the one hand, and the infective groups on the other Are you more likely to find a depleted adrenal in the infected types?

CURRIE: Infection alone does produce it; there's no question about that. In lobar pneumonia, for example, or peritonitis, marked degrees of lipid depletion of the adrenal cortex may occur.

COPE· Is it greater than in the traumatic types?

CURRIE. As for example in non-infected burns? No, I don't think there is necessarily any difference between them.

HARRIS I was wondering, Dr Currie, if you have any data on the state of the glands at the beginning of the "experiment" or the beginning of the stress Dr. Sayers, as I remember, described various changes in different groups of rats exposed to different types of stress. I wondered if you had a group of patients that died suddenly so that you could have a baseline, as it were, from which your observed changes started.

CURRIE· We have not examined the glands of many cases of sudden death. In Scotland these cases have to be reported to the Procurator-Fiscal and the Police make enquiries before a post-mortem is sanctioned. The result is that twenty-four hours have usually elapsed before the case is cleared—and we require fresh material for our investigations. We have, however, examined glands from some patients dying suddenly as a result of multiple injuries and of coronary thrombosis. We have also

examined the adrenal glands removed surgically from cases of hypertension. Zamcheck and Stoner and his co-workers have published their findings in healthy subjects dying suddenly as a result of traumatic injury.

They find that if you take out the adrenal of a rat and extract it immediately you get practically nothing at all. If you let it stand at room temperature for one hour, then the characteristic compound pattern begins to appear qualitatively. If the glands are incubated at 37°C for four hours the full pattern appears. Perhaps your Procurator-

at death, but with the amount actually present at the moment of fixation.

the secretion. On the other hand, in fractures the increased excretion lasted ten days to two weeks; again if they were infected it went on longer. So in severe burns it can be a matter of weeks (in so far as this evidence is valid) before the increased secretion comes back to normal. That would agree very well with your findings.

SOFFER: I should like to ask a question concerning this apparent difference in pattern between patients who have died rather suddenly and those who have been subjected to prolonged periods of stress. I refer particularly to the character of the adrenals of patients who have been treated with ACTH over a prolonged period of time and have

showed a slight increase in size and none showed any change in the lipid distribution. Is this to be expected?

CURRIE: We haven't a great deal of experience of cases who have had prolonged treatment with ACTH and I would not like to comment on your findings. It is perhaps of interest that the ++++ lipid-depleted adrenals without cortisone or ACTH treatment may be divided into two types: some of them present a picture not unlike that seen after a relatively short course of ACTH; while others resemble the adrenals after cortisone treatment. I wouldn't like to say too much about this, because I have been largely responsible for the pituitary part of this study, while Dr. Symington has been largely responsible for the adrenal.

METABOLIC EFFECTS OF ADRENALECTOMY IN MAN*

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Ben May Laboratory for Cancer Research and the Department of Medicine, The University of Chicago, Chicago, Illinois.

TOTAL bilateral adrenalectomy in man has been performed by our group for the control of certain hormone-dependent neoplasms in man and for Cushing's syndrome secondary to bilateral adrenal hyperplasia. The adrenal gland has been shown to influence profoundly the growth of certain animal tumours, in some cases producing acceleration and in others retardation of growth of these neoplasms (Huggins, Bergenstal, and Cleveland, 1953). Certain cancers retain so closely the properties of the cell from which they arose that if the original cells were functionally dependent on hormones the cancer cells will frequently possess these properties. This is seen in certain neoplasms of the prostate in men and of the breast in men and women.

Beatson (1896) discovered that the ovary was a sustaining factor in mammary cancer and oophorectomy has been shown to produce in some cases regression of mammary cancer (Lett, 1905; Horsley, 1947). Huggins and Hodges (1941) clearly pointed out the hormonal dependence of cancer of the prostate and showed that castration and (or) œstrogens could produce profound regression of this neoplasm. The beneficial use of androgens and œstrogens for the control of breast cancer further illustrates the effects of hormonal substances on neoplastic growth (Adair and Herrmann, 1946; Haddow, Watkinson and Paterson, 1944).

*This study was aided by grants from the Jane Coffin Childs Memorial Fund for Medical Research, the American Cancer Society on recommendation of the committee on Growth of the National Research Council, and the Damon Runyon Memorial Fund for Cancer Research.

The adrenal gland in man is capable of producing besides the corticosteroids, hormones which have œstrogenic and androgenic properties. Thus if the control of œstrogenic or androgenic dependent neoplasms is dependent on the complete removal of all sources of steroid hormone production, it will be necessary to remove surgically or to eliminate in some manner the function of the adrenals and gonads. Huggins and Scott in 1945 performed total adrenalectomy for cancer of the prostate which had relapsed following a remission produced by castration. They were unable to evaluate fully this procedure because of the presence of adrenal insufficiency. This problem of adrenalectomy was re-evaluated when the adrenal hormones, cortisone and hydrocortisone, became available.

This paper will present some of the observations made on the metabolic effects of adrenalectomy and the inhibition of human mammary and prostate cancer by adrenalectomy.

Metabolic Effects of Adrenalectomy

Pre- and Post-operative Hormonal Substitution Programme

The purpose of an adequate hormonal substitution programme is to provide adrenal hormones in such concentrations that adrenal insufficiency does not develop during surgery or in the post-operative period. This is accomplished by the use of rather high doses of cortisone and DCA. We have used the same substitution programme developed in 1951 (Huggins and Bergenstal, 1951) and have been able to perform more than 250 adrenalectomies without the development of post-operative adrenal insufficiency. The patient with metastatic carcinoma is usually in a state of poor nutrition and hydration and every attempt is made to correct this as well as possible before surgery (Huggins and Dao, 1952). With good routine post-operative care the patient usually recovers rapidly and within a week is on the future maintenance dose of cortisone and salt.

The hormone substitution programme as well as the excretion of 17-ketosteroid and Pettenkofer chromogens is shown in Fig. 1. This patient had previously had castration for carcinoma of the prostate and pre-operatively had 17-ketosteroid levels of 8 mg. per twenty-four hours and Petten-

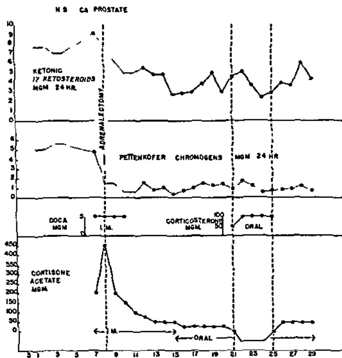


FIG. 1. Urinary steroid excretion following adrenalectomy.

kofer chromogens of 5 mg. per twenty-four hours. Following adrenalectomy the 17-ketosteroids dropped to levels of 3–4 mg. and this most probably arises from the cortisone. Hudson *et al.* (1953) have shown that adrenalectomized patients maintained on glycyrrhizen only excrete essentially no 17-ketosteroids. The Pettenkofer chromogens are presumably of adrenal origin and consist for the most part of dehydroepiandrosterone and its hydrolytic artifacts (Landau, *et al.*,

1951). These chromogenic substances drop to insignificant evels in the urine following adrenalectomy.

Long-term Maintenance Therapy

Our criteria for adequate replacement are: freedom from all signs and symptoms of adrenal insufficiency, with normal salt, carbohydrate and blood pressure balance. This can be accomplished in at least 90 per cent of the cases by the use of cortisone acetate or hydrocortisone by mouth and added dietary salt. A dose of 37·5–50 mg. of cortisone acetate or 20–30 mg. of hydrocortisone per day will accomplish the replacement (Bergenstal and Dao, 1953). The patients are well able to withstand the everyday stresses of living and have a sense of well being.

We have used other steroid hormones for replacement purposes and these are shown in Table I.

Some of the early signs and symptoms of inadequate hormonal replacement are the loss of sense of well being, nausea,

Table I

ADRENAL HORMONES SUPPORTING LIFE

Steroid	*Daily Dose*	*Results*
Hydro-cortisone "Compound F"	20-30 mg. oral	Maintains life with excellent control of salt, blood pressure, carbohydrate balance . sense of well being . normal water diuresis
Cortisone	37½–50 mg oral	Similar to Compound F
DOCA	3–5 mg i m.	Maintains life, blood pressure, Na^+ and K^+ balance . loss of sense of well being . Addisonian pigmentation develops does not correct carbohydrate defect.
Compound S	25–50 mg. i.m.	Maintains life but with borderline insufficieny . poor salt and carbohydrate balance . blood pressure lowered . weakness . Anorexia
Compound B	50–100 mg. oral	Maintains life, blood pressure, Na^+ and K^+ balance

anorexia, weakness and dizziness on standing. Many of these symptoms may be present without any observed changes in blood pressure, serum electrolytes and blood sugar. These symptoms can be corrected by changes in amounts of hormones or salt. An interesting example of a type of inadequate replacement that has been seen in approximately 10 per cent of our patients is characterized by complaints of anorexia, weakness, nausea and dizziness on standing; while when lying these symptoms disappear. This syndrome may occur at any time from a week to many months after the operation. In the majority of these cases the electrolytes and blood sugar are within normal range; however, there is found a marked orthostatic hypotension and orthostatic tachycardia. The attempts to correct these symptoms by increasing the dose of cortisone to 100–150 mg. cortisone a day, has been in most cases unsatisfactory. The patient no longer has marked symptoms of orthostatic hypotension but it is obvious that dose levels of this magnitude would soon produce toxic side effects. It was found that small doses of DCA would quickly correct the orthostatic hypotension and its accompanying symptoms. This orthostatic hypotension syndrome is shown in Fig. 2. The long-acting DOC trimethylacetate given in doses of 30 to 50 mg. once every six to eight weeks is usually adequate to correct the orthostatic hypotension.

While cortisone acetate by mouth is usually adequate for complete hormonal replacement, there are some patients who develop the orthostatic hypotension syndrome, some who are not intelligent enough to follow fully instructions regarding medication, or some who live long distances from their physician; and these patients are usually given monthly injections of from 30—50 mg. trimethylacetate, intramuscularly. This we feel gives a certain safety factor against the development of adrenal insufficiency.

Carbohydrate and Water Balance

The clinical and laboratory findings of hypoglycæmia and flattened oral glucose tolerance curve frequently seen in

patients with Addison's disease maintained on salt and DCA, were not observed in over a hundred cases of adrenalectomized patients being maintained on cortisone acetate. Patients who are on adequate hormonal maintenance with cortisone have normal and essentially the same type of oral and intravenous glucose tolerance tests in the pre- and post-operative periods. There have been no hypoglycæmic episodes after

PULSE	LYING	100	100	90	110	90	80	80	84
	STANDING	160	140	120	160	100	90	88	88
BLOOD	K	5.0	MEQ/L	44		4.0			46
	NA	138	MEQ/L	138		139			140

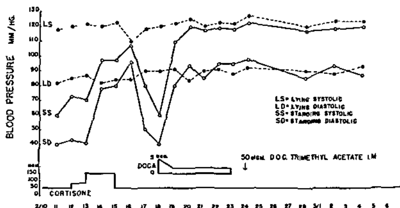


FIG. 2. Correction of orthostatic hypotension with desoxycorticosterone.

intravenous glucose tolerance tests. Prolonged starvation has not produced symptomatic hypoglycæmia. The intravenous insulin tolerance test was essentially the same in pre- and post-operative periods (Fig. 3). Two patients with diabetes mellitus required the same insulin dosage after adrenalectomy while on a maintenance dose of 50 mg. oral cortisone acetate daily. One of these diabetic patients developed a bladder and kidney infection and this was associated with increase in severity of the diabetes and increase in insulin requirement although the maintenance dose of cortisone was unchanged.

By use of the Robinson-Kepler-Power water test (Levy, Power and Kepler, 1946) it is possible to demonstrate a failure of adequate water diuresis in the patients with Addison's disease. A group of patients were given water loads before and after adrenalectomy and all the patients were maintained on 50 mg. oral cortisone acetate daily. As can be

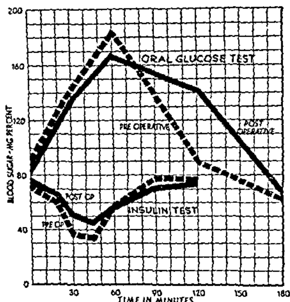


FIG. 3. Glucose tolerance and insulin tolerance tests before and after adrenalectomy.

seen in Fig. 4, there was observed no failure of adequate water diuresis after adrenalectomy while the patient was being maintained on cortisone.

Although nitrogen balance studies have not been done, it is quite clear that at maintenance levels of 37·5–50 mg. of cortisone daily the patients are able to synthesize and to increase the concentration of plasma proteins.

Many clinicians have observed a decrease in the pigmentation seen in Addison's disease following the administration

patients with Addison's disease maintained on salt and DCA, were not observed in over a hundred cases of adrenalectomized patients being maintained on cortisone acetate. Patients who are on adequate hormonal maintenance with cortisone have normal and essentially the same type of oral and intravenous glucose tolerance tests in the pre- and post-operative periods. There have been no hypoglycæmic episodes after

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	STANDING	160	140	120	160	100	90	88	88
BLOOD	K	50 MEQ/L	44		40				46
	NA	138 MEQ/L	138		139				140

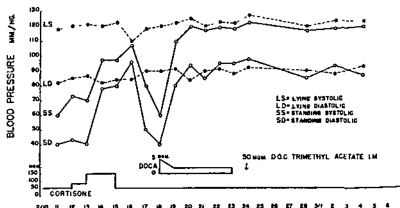


FIG. 2. Correction of orthostatic hypotension with desoxycorticosterone.

intravenous glucose tolerance tests. Prolonged starvation has not produced symptomatic hypoglycæmia. The intravenous insulin tolerance test was essentially the same in pre- and post-operative periods (Fig. 3). Two patients with diabetes mellitus required the same insulin dosage after adrenalectomy while on a maintenance dose of 50 mg. oral cortisone acetate daily. One of these diabetic patients developed a bladder and kidney infection and this was associated with increase in severity of the diabetes and increase in insulin requirement although the maintenance dose of cortisone was unchanged.

spectrophotometer, have observed significant melanin deposition in the skin of all the adrenalectomized patients reported. Skizume *et al.* (1954) report that the melanophoric hormone is increased in the urine following adrenalectomy and increased following the withdrawal of cortisone in adrenalectomized patients. It is of interest that a female with Cushing's syndrome secondary to adrenal hyperplasia, following adrenalectomy with maintenance on 37·5 mg. cortisone, showed no evidence of pigmentation until six months after adrenalectomy, when over a period of two weeks she developed intense Addisonian pigmentation which has persisted for two years.

In 47 cases the range of weight for single adrenal glands was 3·1–5·3 g.; the average weight with standard deviation was $4{\cdot}2 \pm 1{\cdot}1$ g. The total adrenal weight (both glands) was 6·5–10·5 g. The weight of the adrenal glands in most cases did not differ from that of normal healthy adults who have suffered accidental death. A discrete accessory adrenal gland was found in one case near the renal pedicle. Metastases of carcinoma were detected in the adrenal glands in 14 patients in this group. The medulla appeared to be the site of localization.

Inhibition of Human Mammary and Prostatic Cancer by *Adrenalectomy*

Prostatic Cancer

Metastatic cancer of the prostate appears in many cases to be a neoplasm whose growth is dependent upon hormonal substances. The striking relief of symptoms and regression of metastatic lesions following castration have been observed by many clinicians. Thus it would seem that this tumour is dependent, not on hypersecretion of testicular hormones, but rather on what appear in most cases to be subnormal amounts, since many of these patients are in the sixth decade of life. All too often there is a relapse of the neoplasm after remission induced by anti-androgenic methods such as

of cortisone. Huggins and Scott (1945) described the development of intense Addisonian pigmentation in two patients in ten to twelve days following bilateral adrenalectomy at a time when only DCA and adrenal cortical extract were available for replacement therapy. No visible Addisonian type of pig-

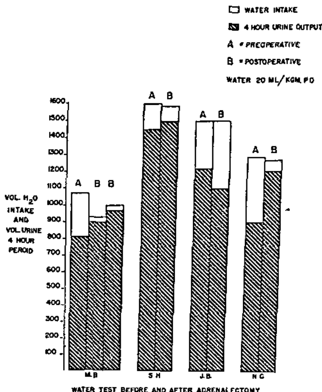


FIG. 4. Water test before and after adrenalectomy.

mentation has been observed in a group of fifty adrenalectomised cancer patients who have been maintained solely on cortisone acetate and sodium chloride. Two patients of this group were later maintained solely on DCA and both developed visible Addisonian pigmentation within two weeks.

Hall, McCracken and Thorn (1953), using a more sensitive method for measuring melanin in the skin with a reflectance

Following relapse many of the patients were given large doses of œstrogen without any observable beneficial effects. Two patients were given 100 mg. of dehydroepiandrosterone intramuscularly daily for ten days and there was a significant increase in bone pain and need for narcotics during administration of this compound. In one case there was slight rise in blood acid phosphatase. Dehydroepiandrosterone has slight androgenic properties and presumably is of adrenal origin.

There seems to be a clinical remission of the disease of considerable magnitude following adrenalectomy; however, the relapse rate is high.

The explanation of the relapse is not known; however, one could speculate as to whether the tumour may become sensitive to minute amounts of androgen derived from the maintenance cortisone or production of androgenic compounds by accessory adrenal tissue which under the stimulus of possibly elevated ACTH blood levels may become functional at a level not sufficient to maintain life but to stimulate growth of the neoplasm. Pearson (1954) reports that cases autopsied and searched carefully for accessory adrenal tissue have shown as high as 30 per cent with accessory adrenal tissue. Also the possibility of the tumour changing its metabolic characteristics so that it is no longer hormone-dependent should be considered.

Cancer of the Breast

Cancer of the breast is not a single pathological state, but consists of categories that differ in clinical and functional characteristics. Some of the neoplasms in both sexes are composed of cells of sufficient functional maturity to undergo a decrease in activity when critical quantities of supporting hormones are removed through surgical removal of these sources; the patient is benefited in this circumstance.

This group of cases will be presented in two parts. one group who had adrenalectomy alone, and the other group who had adrenalectomy and oophorectomy at the same time.

castration and (or) œstrogens. Adrenalectomy has been performed in this group of patients in an attempt to remove other possible sites of androgen hormone formation (Huggins and Bergenstal, 1952).

The patients were all suffering from advancing metastatic carcinoma of the prostate with lesions in bones and soft tissues. Our criteria for objective response were decrease in size of palpable tumour masses, halt in progression of osteolytic and osteoblastic lesions, relief of severe bone pain, weight gain, increased hæmoglobin synthesis, and ability to return to their usual activities. Not all patients showed all these changes. Three patients with elevated acid phosphatase in blood showed a return to normal values. In two of these cases a relapse was preceded by elevation of the acid phosphatase. One of the patients with a serum acid phosphatase of 90 units/100 ml. had a fall to 3 units/100 ml. which has persisted for thirty-three months, and the patient is still well and with no signs or symptoms of relapse. There is frequently observed a rise in alkaline phosphatase indicating osteoblastic activity.

Table II
PROSTATE CANCER (22 CASES)
Adrenalectomy for metastatic carcinoma of prostate after relapse from remission induced by gonadectomy

Objective response 9 cases	*No response 13 cases*
Per cent objective response 41 per cent	13 cases all dead

Table II shows the results obtained in 22 cases of cancer of the prostate. There was observed an objective response in 9 cases (41 per cent) which persisted for an average of 13 months before relapse occurred.

Six patients complained of moderately severe hot flushes very similar to those they had noted following castration.

It is most difficult to evaluate ovarian function in terms of ability to secrete hormonally active compounds after the menopause, whether this be spontaneous or induced by X-ray sterilization and (or) androgens. Many of the biological and chemical tests are insufficiently sensitive clearly to eliminate

Table III

BREAST CANCER (37 CASES)

Bilateral Adrenalectomy alone for progressive metastatic carcinoma of the breast

Objective response 14 cases (38%)	*No response 23 cases*
Alive 10 cases Duration of remission 6 to 36 months *Average* 19 months	*Dead* 23 cases Duration of life after adrenalectomy 1 to 14 months. *Average* 5 months
Dead 4 cases Duration of remission 8 to 13 months *Average* 9 months	
Status of Ovarian Function *Alive* 10 cases 5 cases spontaneous menopause 5 cases surgical castration *Dead* 4 cases 1 case spontaneous menopause 1 case surgical castration 2 cases X-ray sterilization	*Status of Ovarian Function* *Dead* 23 cases 10 cases spontaneous menopause 6 cases surgical castration 7 cases X-ray sterilization

possibilities of continuing ovarian function. Dao (1953) has investigated this problem using the bioassay technique for œstrogen excretion in the urine. He has found that the normal and cancer patients secrete significant amounts of œstrogen in the spontaneous post-menopausal period and that œstrogen secretion in the X-ray castrated woman was more than in the spontaneous menopausal group. Following ovariectomy and adrenalectomy the œstrogen excretion was zero.

Bilateral Adrenalectomy Alone:

This group consisted of 37 cases: two male mammary cancer and 35 female mammary cancer. There was no attempt at selection of cases except that it was thought these patients had no significant ovarian function. All the patients had had some type of previous therapy, either hormonal and (or) X-ray, but had relapsed and growth of their neoplasms was advancing rapidly. The rate of growth of the tumours was measured either directly, if they were accessible, or by indirect methods such as roentgen-ray examination and laboratory tests if the tumours were inaccessible. Regression of soft tissues metastases consisted of the disappearance of local recurrent lesions, the disappearance of metastatic pleural and pulmonary lesions, involution of metastatic lymph nodes and healing of cancerous ulcers. Regression of metastatic bone lesions was demonstrated by the healing of pathological fractures and of osteolytic defects, and by characteristic changes in serum alkaline phosphatase (Huggins and Dao, 1953). Subjective improvement in this group of patients consisted of disappearance of intractable bone pain, disappearance of respiratory symptoms, the recovery of a sense of well being, increase in appetite, gain in weight and rec[illegible]ing of strength and vitality.

The results of adrenalectomy alone in this group of 37 cases are shown in Table III. The objective response was observed in 38 per cent of the cases. Of the 14 cases showing objective response, 10 are alive, with the duration of remission from six to thirty-six months, with an average of nineteen months. Four of the patients have relapsed and have died; their average duration of remission was nine months. In contrast, the 23 cases showing no effects had an average duration of life after adrenalectomy of five months. The ovarian status of the patients is also shown in Table III. All the patients had not menstruated or had had an artificial menopause long enough before adrenalectomy so that it was felt this did not contribute to the objective response observed after adrenalectomy.

which sustains mammary cancer and propagates the disease. Other types of mammary cancer fail to respond to adrenalectomy. One of the urgent problems at the present time is the proper selection of cases for this operation.

Table IV

BREAST CANCER (60 CASES)

Bilateral adrenalectomy and oophorectomy for progressive metastatic carcinoma of the breast

Objective response 27 cases (45%)	*No response 33 cases*
Alive 17 cases Duration of remission 6 to 26 months *Average* 11 months *Dead* 10 cases Duration of remission 6 to 24 months *Average* 12 months	*Dead* 33 cases Duration of life after adrenalectomy and oophorectomy 1 to 10 months *Average* 4 months
Status of Ovarian Function *Alive* 17 cases 6 cases spontaneous menopause 4 cases pre-menopause 5 cases X-ray sterilization 2 cases androgen-induced menopause *Dead* 10 cases 2 cases spontaneous menopause 5 cases pre-menopause *3 cases X-ray sterilization*	*Status of Ovarian Function* *Dead* 33 cases 9 cases spontaneous menopause 9 cases pre-menopause 14 cases X-ray sterilization 1 case androgen-induced menopause

In a review of a series of 100 consecutive unselected cases of recurrent far-advanced cancer of the breast an attempt has been made to set up certain criteria which will make possible selection of cases that will show a satisfactory response. Huggins and Dao (1953) have discussed some of the characteristics of steroid-dependent mammary cancers in a previous paper.

Bilateral adrenalectomy and oöphorectomy:

It is certain that the ovary is strongly implicated in the maintenance of mammary cancer in the human female, although the relative function of each of the ovarian hormones in this regard is not well understood. The involution of mammary cancer after oophorectomy is clearly due to removal of hormones found in the ovary; the most reasonable explanation of the beneficial effects of adrenalectomy is the elimination of hormones of a similar type. The excretion of œstrogen in the urine of women who have undergone oöphorectomy is abolished by adrenalectomy. Since there is a dual supply of hormones of ovarian type, both sources should be eliminated in cancer of the breast when they contribute importantly to the endocrine pool. Regression of mammary cancer after deprivation of steroids by surgical means is a function of removal of critical amounts of sustaining hormones, together with sufficient maturity of the neoplastic cells so that they are dependent on hormones—it depends on hormone production, reactivity of the cells and tumour-host relationship.

The group of 60 patients (Table IV) who had both adrenalectomy and oophorectomy suffered from rapidly advancing metastatic carcinoma of the breast. These patients in many instances had had previous types of therapy which had given temporary or no benefit and their cancers were progressing. Of this group of 60 cases, 45 per cent (27 cases) obtained objective response, while 33 cases had no response. The duration of remission has been six months to twenty-six months with an average duration of eleven months. The cases that failed to respond had a duration of life after adrenalectomy from one month to ten months with an average duration of life of four months.

The ovarian status of these patients is shown in Table IV.

Gratifying relief of patients with disseminated mammary cancer often follows adrenalectomy and this observation has been confirmed by other investigators (Pearson, *et al.*, 1954), (Taylor, *et al.*, 1953), (Krieger, *et al.*, 1953), (Randall, 1954). These cases indicate the presence of an adrenal component

which sustains mammary cancer and propagates the disease. Other types of mammary cancer fail to respond to adrenalectomy. One of the urgent problems at the present time is the proper selection of cases for this operation.

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Briefly they are: (1) Age. The most favourable results :urred in women between ages of 40 and 65 (Table V.);) Degree of malignancy. It would appear that the best :asurement of the growth rate in a case of breast cancer is

Table V

AGE IN RELATION TO A FAVOURABLE RESPONSE TO ADRENALECTOMY

Age	*Total No. of cases*	*No of cases with favourable response*
Under 40	13	3
40–49	42	19
50–59	31	12
60–69	8	4
70– up	1	0

Table VI

PIDITY OF TUMOUR GROWTH IN RELATION TO A FAVOURABLE RESPONSE TO ADRENALECTOMY

Time interval between Mastectomy and onset of Metastasis	*Total No of cases*	*No of cases with favourable response*
Under one year . .	28	3
1–2 years .	29	14
2–5 years . .	30	16
Above 5 years .	8	5

e time interval between the radical mastectomy and the iset of metastases. The better responses to adrenalectomy ccurred in women who had a prolonged interval between astectomy and the recurrence of metastases of the cancer Table VI.); (3) Specificity of the tumour. Adrenal-responsive imours can be recognized in many cases by histological camination of the primary tumour. The adenocarcinoma we el is the most specific functional category of mammary cancer.

(4) High titre of œstrogenic substance in the urine. Table VII shows a series of responsive and non-responsive tumours with the histological type of neoplasm and the œstrogenic excretion in the urine. The responsive group has an œstrogen excretion approximately three-fold higher than the non-responsive group.

Pearson, *et al.* (1954) have described two other criteria of œstrogen-dependent mammary cancers. First, an increased excretion of calcium in the urine following the administration of œstrogenic substances to patients with osseous metastases indicates an œstrogen-dependent cancer. Secondly, patients who have gained a remission from oöphorectomy commonly respond favourably to subsequent adrenalectomy. It is obvious that the second consideration is inoperative when the ovaries are not functioning.

Conclusions

Total bilateral adrenalectomy can be performed in man with little danger. The adrenalectomized patient can be well maintained in good health with adrenal cortical hormones.

The growth of certain hormone-dependent neoplasms in man can be inhibited by the removal of the sources of the sustaining hormones.

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ŒSTROGEN EXCRETION

Responsive Mammary Cancer				
Case	Age	Cause of Menopause	Oestrogen Titre i u /24 hr	Histolog
M.W.	45	Surgical	38·1	Adeno-(
C.K.	60	Spontaneous	16·0	Adeno-(
A.M	52	Spontaneous	15 0	Duct-C.
M.A.	52	Irradiation	58·9	Adeno-(
Z.S.	45	Irradiation	16 4	Papillary
A.M.	32	Surgical	18 6	Adeno-CA. and
A B.	49	Spontaneous	11 7	Adeno-(
V.R.	47	Surgical	16 0	Adeno-C
Y.S	59	Surgical	22 0	Adeno-C
L.W.	60	Spontaneous	8 8	Scirrhous Ade
Total cases 10 Median 16 0 i u /24 hr.				

One Internat

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/II

N WOMEN WITH CANCER OF BREAST

Unresponsive Mammary Cancer				
Case	Age	Cause of Menopause	Oestrogen Titre i u /24 hr	Histology
M Q.	57	Surgical	9·6	Undiff.-CA.
L.P.	45	Irradiation	4·7	Undiff.-CA.
V C.	47	Irradiation	12 3	Undiff.-CA.
M.S.	36	Irradiation	3 7	Undiff.-CA.
K.A.	49	Irradiation	9 2	Adeno-CA.
M.S.	50	Irradiation	6 7	Undiff -CA.
A.S.	57	*Spontaneous*	*9·0*	*Undiff.-CA.*
G.S	50	Surgical	12 0	Undiff.-CA .
E.Z.	31	Surgical	8·4	Undiff -CA.
J.D.	29	Surgical	8·4	Undiff.-CA.
Median 8 4 i u./24 hr.				

equivalent to 0 1 μg of Œstrogen

SKIZUME, K., LERNER, A., and FITZPATRICK, T. (1954) Abstract, 36th Meeting of the Endocrine Society.
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DISCUSSION

BAYLISS: We have also seen the orthostatic hypotension, which you observed, after adrenalectomy. Have you done any studies on the blood volume in these patients.

BERGENSTAL. No, but we plan to measure the blood volumes of this group with iodine-labelled albumin.

SPENCE: May I ask a question about mammary cancer? It is suggested that the improvement which takes place is due to the removal of œstrogenic hormones. In that case I don't understand the reports of improvement in mammary cancer in older women treated with stilbœstrol.

BERGENSTAL: This is a very important problem, and I wish I knew the answer. We certainly feel that there is more involved than just the œstrogenic hormones. It may be another ovarian and (or) adrenal type of hormone, and certainly we feel, from some work we are just doing on luteotrophin, that there may be many important interrelationships between the pituitary and these hormones of the adrenal and ovary contributing to the continued growth of the tumour. I certainly don't understand why some of these post-menopausal older women get a satisfactory response to œstrogen unless there is a pituitary suppressive effect.

PRUNTY: I feel that what one so badly lacks in this kind of work is control material. What we need to know is the prognosis of the selected cases that you think would benefit. One of the criteria that you brought up was the long interval between mastectomy and bone metastases. It is my impression that those are the people who tend to survive rather well in any case.

BERGENSTAL. Yes, in many cases they do.

FORSHAM: We have helped Dr. M. Galante on a series of more than 35 cases of bilateral adrenalectomy and oophorectomy, with results that are analogous. Our chief problem is osteoporosis. After about eight months or so these patients begin to get bone pain, but this time it is not due to metastases, but to progressive thinning of the bone. I wondered what you do about it.

blocking other catabolic actions. Osteoporosis becomes an increasingly serious problem the more successful you are with the operation in terms of survival.

BERGENSTAL: Have you tried strontium?

FORSHAM: Not to any extent in these patients. Dr. E. Shorr found that strontium would settle in the bone in addition to calcium, and could make up an extra 10 per cent of bone osteoporosis.

THORN: These cases you speak of were maintained on 50 mg. of cortisone a day?

FORSHAM. Ours are getting 37·5, but that still favours osteoporosis.

BROWNE: I should like to ask Dr. Bergenstal, if you remove the adrenals in Cushing's syndrome and maintain the patients on 50 mg. of cortisone daily, after a period of time does their osteoporosis improve?

BERGENSTAL: Yes, but it takes about a year or two years.

FORSHAM. I should like to report on some preliminary work: Dr. M. Galante in our Department of Surgery has tried to eliminate the œstrogens, following the work done by Dr. Biskind in rats, in which

adrenal was removed in four patients, and the right adrenal was kept *in situ* (we did certain tests which I shall tell you about in a minute). In a

her disease, and is still alive now a year after the procedure. Before surgery, when we stimulated by ACTH tests, her hydrocortisone-like

transplanted the adrenal there was a definite, measurable increase in œstrogens on ACTH, but there was absolutely none demonstrable after this transplantation. The biological proof for the virtual absence of œstrogens is given by the fact that this woman has had a complete remission. There are three other cases, one failure in anastomosis, one too early and one other is doing well. So we have here a possibility, by utilizing the different rates of detoxification of œstrogens by the liver, to carry patients without any substitution therapy.

BROWNE. As so often is said in discussion "This reminds me of some work I did" in 1931 on infantile animals. I took out their ovaries and implanted them into the spleen. They did not develop tumours but they developed enormous ovaries, practically filling the spleen and entirely composed of corpus luteum tissue. Progesterone, from other evidence, is also detoxified in the liver.

LUFT: The Swedish results with bilateral adrenalectomy are similar to those of Dr. Huggins and Dr Bergenstal. I should like to ask some questions firstly, did you examine the bone tissue after adrenalectomy?

BERGENSTAL: No, not in this group. We did in some of the autopsy cases.

LUFT. Did you find any cancer tissue?

BERGENSTAL: We found a peculiar type of cell, which we have described before, which looks as if it were undergoing pyknosis and degenerative changes, with a densely calcified bone around the metastasis.

LUFT. I have been told by Dr. Frankson in Stockholm that when the pathologist examined the metastases in the bone in a case after

may be expected ...
the breast or prostate?

CURRIE: Is there any evidence available as to the histological appearance of the pituitary in these cases with bilateral adrenalectomy?

BERGENSTAL: Dr. Gomori is working very hard on that, but I haven't any results as yet.

CURRIE: Dr. Bergenstal made the point that the histological picture in the tumours varied from one area to another, and I would be in full agreement with that. It is obviously of importance to a pathologist to ... bilateral ...

very ...
and if one looks at a ...
spheroidal-cell type ...
tion. There is no sharp dividing line between the histological ...
breast cancer ...

and ...

THORN: Wouldn't ...
most suitable cases ...
tomy and relapses, ...
that two- to three-year period. You ...
... biopsy of two years ago.

hist...
tact...
carcinomas and ...

SCOWEN: Have you any data about the character of the vaginal smear?

BERGENSTAL: No. We have used bioassay of urinary œstrogens instead.

FORSHAM: We have done vaginal smears and found this very unsatisfactory: 90 per cent of the smears come back with "slight œstrogenic effect" in the oöphorectomized, adrenalectomized patients and we don't know what that means.

SCOWEN: I ask the question specifically because there are some patients who have had both ovaries and adrenals removed and who still have a very active œstrogen smear from the vagina. I can't say in all cases, but in some I know it is not due to adrenals left behind, because

monas, might give you that kind of smear?

SCOWEN: No, the smear is quite different

THORN: May I make this suggestion? In the prostatic cases with adrenalectomy you have three factors. one is the removal of adrenal

to compare with your operated cases?

BERGENSTAL: We have done just that, and found there is little or no effect on acid phosphatase or on bone pain, or on general course. In fact many of these patients, before they had their operation, went through exactly the same replacement therapy, high doses of DCA, cortisone, everything except the operation, and there was no satisfactory response

adrenalectomy and ovariectomy in January this year. She had a fungating mass nearly the size of a soup-plate from a breast removed twenty-six years ago I had removed the opposite breast during the war. Within three days of adrenalectomy and ovariectomy she said all her bone pains had gone. The whole mass has now skinned over most dramatically. I feel this is an experiment that must go on, but I think we want a clearer lead from you as to the type of case which will benefit

culous bronchopneumonia.

THERAPEUTIC RESULTS OF HYPOPHYSECTOMY IN METASTATIC CARCINOMA OF THE BREAST AND IN SEVERE DIABETES MELLITUS; ADRENOCORTICAL FUNCTION AFTER HYPOPHYSECTOMY*

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OUR first attempt to perform a complete hypophysectomy was made in July 1951 (Luft *et al.*, 1952; Luft and Olivecrona, 1953). We have since made 80 hypophysectomies in different diseases. The present report concerns the two main groups submitted to this procedure, metastatic carcinoma of the breast and severe diabetes mellitus. In addition some data on the adrenal cortical function in hypophysectomized patients are given.

Metastatic Carcinoma of the Breast

Up to February, 1954, 37 cases of metastatic carcinoma of the breast had been hypophysectomized. Almost every case showed widespread metastases, and the patients had been treated in different ways before the hypophysectomy. Four of the early cases had incomplete removals of the hypophysis, and three died within one month after the operation from cerebral embolism, pulmonary embolism and coronary thrombosis, respectively. Thus, there remain 30 cases to be discussed. Of these 30 cases, 16 are dead and 14 are alive, from six months to two-and-a-half years after the hypophysectomy.

*These studies were aided by grants from the Medical Research Council of Sweden, Knut and Alice Wallenberg's Foundation and the Swedish Diabetic Association.

All seven cases presenting brain metastases and (or) liver metastases of such an extent that an enlarged and firm liver could be palpated, have died, except one. If these cases, too, are subtracted, *there remain 23 cases, of which 13 are alive and 10 are dead.* Of the 13 living patients, two are men with metastatic carcinoma of the breast.

Four of the patients had scirrhous cancer and all these belong to the living group. The other surviving patients are equally distributed between the group with cancer of a low differentiated type and the group with adenocarcinoma.

All women above sixty years of age—four cases in all—have died without showing any influence of the operation on the course of the disease. Except for this, the survivals are equally distributed among the different age groups. Two men were operated on. They were both over sixty and have reacted favourably.

Of the sixteen deaths, six occurred in patients already having extensive liver or brain metastases before the hypophysectomy, and four in women above sixty years of age. Of the remaining six deceased cases, one died of adrenal cortical insufficiency and five of progression of the cancer.

The effect of hypophysectomy has also been evaluated with regard to the effect on pain, on local invasive growth, and on the metastases in the lymph nodes, lungs, pleura and the skeleton.

Table I shows the effect on pain. The pain disappeared in eight out of ten of the surviving patients, was ameliorated in one case and unchanged in the remaining one. A transient decrease in pain was seen in some of the deceased patients.

Table II shows the effect of hypophysectomy on local invasive growth and metastases in the lymph glands. A *marked improvement* was obtained in all of the surviving cases except one, and in two of the cases that died. Of the two latter cases, No. 8 is worth special attention. This patient showed a remarkable disappearance of all soft tissue metastases but died eight months after the operation in adrenal cortical failure precipitated by an allergic reaction with

high body temperature, during which the maintenance dose of cortisone of 25 mg. per day was not increased.

Table III gives the changes in the lung and pleural metastases observed *after* hypophysectomy. A disappearance or decrease of these metastases could be seen in most of the surviving cases.

Table I

EFFECT OF HYPOPHYSECTOMY ON PAIN IN METASTATIC MAMMARY CANCER

Case No	Time of observation months	Pain	
		Before operation	After
1	28	——	——
5	20	——	——
11	14	+	Disappeared
13	14	——	——
15	13	++	Disappeared
16	13	+	Disappeared
17	13	(+)	Disappeared
21	12	+	Disappeared
25	7	++	Unchanged?
26	7	——	——
27	7	+++	Disappeared
28	6	+++	Disappeared
29	3	+	Disappeared
30	19	+++	Disappeared
2	17	+	Disappeared for 15 months
3	1	+++	Unchanged
4	2	+++	Unchanged?
6	3	+++	Disappeared
7	10	——	
8	9	+	Disappeared for eight months
9	5	++	Decreased during first month
10	10	++	Decreased
12	10	++	Disappeared for one month
14	4	++	Decreased
18	9	+	Decreased during first month
19	2	+	Unchanged
20	2	+	Transient decrease
22	3	+++	Decreased
23	3	+	Increased
24	3	——	

Table II

EFFECT OF HYPOPHYSECTOMY ON LOCAL INVASIVE GROWTH AND LYMPH GLAND METASTASES IN METASTATIC MAMMARY CANCER

Case No	Time of observation in months	Before Hypophysectomy								After Hypophysectomy
		Skin		Involvement of Lymph Glands				Arm		
				Homolateral		Contralateral				
		Infiltr	Ulcerat	Axillary	Supraclav	Axillary	Supraclav	Œdema	Restrict. of movement	Condition of Local changes
1	28	+++	+++	?	?	++	++	+++	+++	Almost disapp. for about 20 months, then recurrencies
5	20	—	—	—	—	—	—	—	—	—
11	14	++	—	+	—	—	—	—	—	Normal findings
13	14	—	—	+	—	—	—	+	—	Normal findings
15	13	—	—	—	—	—	—	—	—	—
16	13	++	+	+++	++	—	—	+++	+++	Improvement of all changes
17	13	+	—	—	? +	—	—	+++	++	Normal local findings, arm less swollen
21	12	—	—	—	+	—	—	—	+	Marked improvement
25	7	—	—	—	++	—	—	—	—	Improvement?
26	7	—	—	—	++	—	—	—	+	Normal findings
27	7	—	—	—	+	—	—	—	—	Normal findings
28	6	—	—	—	—	—	—	—	—	—
29	8	—	—	—	—	—	—	—	—	—
30	19	—	—	—	—	+	—	—	—	Normal findings
2	17	++	—	? +	? +	—	—	+	+	Progress
3	1	+++	—	+++	+++	—	+++	+++	+++	Progress
4	2	—	—	—	—	—	—	—	—	—
6	3	—	—	—	—	—	+	—	—	No improvement
7	10	++	+	+++	—	—	—	—	—	No improvement
8	9	—	++	+ +	+++	++	+++	—	+	Disappeared
9	5	—	—	—	+	—	—	+	—	Progress
10	10	—	—	—	+	—	—	—	—	No improvement
12	10	—	—	++	—	—	—	+	+	Improvement for 5 months
14	4	—	—	—	—	—	—	—	—	—
18	9	+	—	—	—	—	—	++	—	No improvement
19	2	—	—	—	—	—	—	—	—	—
20	2	+++	+++	—	+++	—	++	+++	+++	Progress
22	3	—	—	—	—	—	—	—	—	—
23	3	+++	+	—	++	—	+	+++	++	Progress
24	3	+++	+++	—	+++	+++	+++	+++	++	Progress

Table III

EFFECT OF HYPOPHYSECTOMY ON LUNG AND PLEURAL METASTASES IN METASTATIC MAMMARY CANCER

Case No	Time of observation in months postoperative	Before operation		After operation	
		Lung Metastases	Pleural exudate	Lung Metastases	Pleural exudate
1	28	+	—	—	—
5	20	—	—	—	—
11	14	—	—	—	—
13	14	+++	—	Disappeared	—
15	13	+++	+	Decrease	+
16	13	—	—	—	—
17	13	—	—	—	—
21	12	—	—	—	—
25	7	+	+++	?	Decrease
26	7	++	—	Decrease ?	—
27	7	—	—	—	—
28	6	—	—	—	—
29	3	++	—	Decrease	—
30	19	—	—	—	—
2	17	—	—	—	+
3	1	+	+	+	++
4	2	+++	++	+++	++
6	3	++	—	++	—
7	10	—	—	—	—
8	9	—	++	—	++ Transient Decrease
9	5	? +	—	? +	—
10	10	—	—	—	—
12	10	—	—	—	—
14	4	—	—	—	—
18	9	—	—	—	—
19	2	++	—	++	—
20	2	+	—	+	—
23	3	? +	—	? +	—
22	3	+	—	+	—
24	3	+	—	+	—

Table IV gives an approximate estimate of the changes in the bone metastases after hypophysectomy. The difficulty in appraising the effect on bone metastases is well known. An increased density makes them more clearly visible on the X-ray picture, and may make metastases visible that could

not be seen earlier. In Table IV we have preferred to denote the metastases as unchanged if they did not show any significant regression or showed only moderately increased density. Minor regressions have not been taken into account.

Table IV

EFFECT OF HYPOPHYSECTOMY ON BONE METASTASES IN METASTATIC MAMMARY CANCER

Case No	*Time of observation in months postoperative*	*Before operation*	*After operation*
1	28	——	——
5	20	——	——
11	14	++	Increase?, incr. density
13	14	——	——
15	13	+++	Increase
16	13	——	——
17	13	+	Unchanged
21	12	++	Increased density
25	7	——	New metastases
26	7	++	Unchanged
27	7	++++	Unchanged
28	7	++	Unchanged
29	3	+++	Unchanged
30	19	+++	Unchanged

Our patients have been given substitution therapy of 25 mg. of cortisone and 50 mg. of desiccated thyroid daily. With this substitution their general condition was remarkably good, and most of the surviving patients were able to return to work.

To summarize: our limited material seems to show that hypophysectomy had a favourable influence on metastatic mammary cancer in women below sixty years of age not presenting brain metastases or very extensive liver metastases. The type of cancer seems to be of minor significance, except for the fact that the scirrhous type has reacted especially well.

Severe Diabetes Mellitus.

Up till now nine cases of severe diabetes mellitus with malignant vascular complications have been hypophysectomized: five women and four men, between 22 and 31

Table

FINDINGS AT THE TIME OF HYPOPHYSECTOMY

Case No	Eyes						Kid
	Vision	*lens turbidity*	*retinal changes*			*albuminuria 0/00*	*NPN*
			exudates	*hæmorrhages*	*proliferations*		
1	dx. amaurosis sin.	dx = sin =	dx. +++ sin +++	dx +++ sin +++	dx +++ sin +++	2	30–40
2	dx. amaurosis sin. perc. of light	dx +++ sin ++	dx ? sin +++	dx ? sin +++	dx ? sin. +++	2	38–50
3	dx 0 5/60 sin almost amaurosis	dx + sin +	dx = sin =	dx = sin =	dx +++ sin. +++	1–3	38–50
4	dx ≦0 1* sin ≦0 6	dx + sin +	dx + sin +	dx ++ sin. ++	dx + sin =	1	37
5	dx almost amaurosis, sin 2/60	dx = sin. =	dx. + sin. +	dx +++ sin +++	dx. ++? sin ++?	2–4	40

*Monoyer's system (normal vision 1 0).

years of age. They had had their diabetes for fourteen to twenty-one years and complications affecting eyes and kidneys for at least one to twelve years. Their age at hypophysectomy was from 22 to 30. Two of these, operated upon about one month ago, cannot be evaluated at present. Of the remaining seven cases, operated upon one to two-and-a-half years ago, four are alive and three are dead. Two of the deaths occurred among the earliest cases, before we had experience with the complications that might occur in this operation. One was a young man who was re-operated for a suspected intracranial hæmatoma some hours after the hypophysectomy. Only a moderate brain œdema was found. He died the day after this second intervention. The second was a young woman, who died suddenly one month after the operation—a few days before she was going to leave the hospital—in a syndrome, with which we later became familiar and which presumably is due to hypoglycæmia with rather unusual features. The last deceased case, a young man

FIVE CASES OF DIABETES MELLITUS

ys		*Cardiovascular*							
GFR l /min.	*RPF ml /min.*	*blood pressure mm. Hg.*	*heart size ml /m²*	*Calcific of large vessels on X-ray*	*serum albumin %*	*serum globulin %*	*hgb / red cells %/mill.*	*Œdema*
75†		175/80-190/110	310	+			63/3 5	+
60†	216	150/105	330	−	3 9	2 7	60/2 8	+
42 57†	261	145/100-170/105	360	−	3 2	3 2	55/3 3	+++
87	386	145/100		−	5 2	1 9	67/3 6	+
68	453	220/110	420	+++	3 6	2 3	68/3 5	+

†creatinine clearance

of twenty-four, was operated on in spite of having very advanced arteriosclerosis. At the operation the brain vessels were found to be brittle, which caused severe bleeding during the operation. He recovered from this but later showed slowly advancing mental deterioration. He finally reached a state of stupor and died from complicating infections.

Table V shows the status of the eyes, kidneys and cardiovascular system at the time of operation. One of the cases (No. 4) had some vision left. Only cases 4 and 5 were considered to have less than 50 per cent reduction of the glomerular filtration rate. All patients had an elevated blood pressure, which was more marked in case 5; this case also showed advanced arteriosclerosis.

As could be expected, the insulin sensitivity increased after hypophysectomy (Table VI) One of the patients can get along well without insulin; the remaining need up to eight units of protamine zinc-insulin per day. The insulin requirement is determined mainly by the maintenance dose of

cortisone; as small a dose as 5 mg. of cortisone markedly increases the blood sugar level and the daily amount of urinary sugar.

Table VI

CHANGES IN INSULIN REQUIREMENT AFTER HYPOPHYSECTOMY IN DIABETES MELLITUS.

Case No	Daily requirement of insulin (units per day)	
	before hypophysectomy	after hypophysectomy
1	40 PZI+20 r.*	8 PZI
2	28 PZI+14 r.	6 PZI
3	24 PZI	0
4	20+16 PZI	8 PZI

*) PZI = protamine zinc-insulin, r = regular insulin

The aim of the operation was to check the progress of the vascular complications. It is at present difficult to appraise the results in this respect. Thus, in only one of the patients some vision remained before the operation, and, at present, one year after the operation the vision is unchanged and th re has been no progress of the eyeground changes (case 4).

The kidney function as measured by clearances is especially difficult to evaluate after hypophysectomy. The clearances of inulin and PAH are presented in Table VII, which shows that they remained unchanged in Case 1 for two-and-a-half years after the operation and decreased in the remaining four. The decrease in Case 4 shortly after the operation followed an attack of acute pyelonephritis, but the clearances have not changed since. However, one has to take into account the fact that hypophysectomy *per se* may cause a decrease in the clearances, especially when the patients are kept on a low substitution. The albuminuria has disappeared in Cases 1 and 4 but persists in the three others.

The blood pressure, which was elevated in all cases, returned to a normal level after the operation.

Table VII

CHANGES IN GLOMERULAR FILTRATION RATE AND EFFECTIVE RENAL PLASMA FLOW AFTER HYPOPHYSECTOMY IN DIABETES MELLITUS

Case No	*Date of test*	*Date of hypo-physectomy*	*GFR ml/min*	*RPF ml/min*	*FF*	*RBF ml/min.*	*BP mm Hg*	*Substitution*†
1.	July 1950	Nov. 1951	152*				140-165/70-105	
	Jan 1951		76*				130-180/90-110	
	Nov 1951		75*				175-190/80-110	
	July 1952		55	342	0 16	526	130/80	DCA+TP+thyroid
	July 1952		40	363	0 14	540	135/80	DCA+TP+thyroid
	Dec 1952		69	438	*0 16*	*730*	*140/100*	DCA+TP+thyroid
	April 1953		63	353	0 18	504	130/90	DCA+TP+thyroid
	Jan. 1954		71	339	0 21	506	130/90	DCA+TP+thyroid
2	Jan 1953	March 1953	60*	216			150/105	
	March 1953		69*					
	April 1953		30	232	0 13	387	140/80	DCA 5 mg
	June 1953		36	197	0 18	308	123/90	DCA+TP+thyroid
	Febr 1954		22	201	0 11	264	140/90	DCA+TP+thyroid
3	Nov 1950	April 1953	42	285	0 15		120/80	
	March 1953		(42)	261	0 16	458	145/90	
	Sept 1953		62	272	0 23	394		Cortisone 5+thyroid
	Oct 1953			373			130/90	DCA+TP+cortisone 5+thyroid
	Febr 1954		35	303	0 10	558	130/90	DCA+TP+cortisone 5+thyroid
	Febr 1954		82	322	0 10	460	130/90	DCA+TP+cortisone 5+thyroid
4	May 1953	May 1953	76	339	0 22	565	143/100	
	Oct 1953		43	188	0 23	813	145/100	DCA+TP+thyroid
	Jan 1954		41	258	0 16	391	115/85	DCA+TP
5.	Nov. 1953	Dec 1953	68	453	0 15	676	220/110	
	March 1954		55	434	0 13	689	160/90	Cortisone 5+DCA+TP+thyroid

* creatinine clearance
† DCA = desoxycorticosterone acetate
TP = testosterone propionate

The maintenance therapy consists of a daily dose of 5 mg. of cortisone in three of the cases (except case 1) and 50 mg. of desiccated thyroid. Testosterone pellets have been implanted in all of them. The first cases were also implanted with DCA pellets. However, DCA gave rise to hypertension and œdema in two cases and has later been discontinued in all except case 1.

To summarize: our material is too small to permit any definite statement about the value of hypophysectomy in diabetes mellitus with malignant vascular complications. The results obtained so far are encouraging and the study is continuing.

Adrenal Cortical Function After Hypophysectomy.

The adrenal cortical function after hypophysectomy was studied by following the urinary excretion of 17-ketosteroids and corticoids (determined by the method of Norymberski, *et al.*, 1953), by the adrenaline test, the changes in insulin sensitivity and the changes in water metabolism.

The excretion of 17-ketosteroids and corticoids during the first month after hypophysectomy was studied in a number of patients, who purposely were not given any substitution after the day of operation. The results obtained in a representative case are shown in Fig. 1. As may be seen, the values for both groups of steroids reached a very low level as soon as the ninth post-operative day, and stayed at almost zero for the remaining period without substitution. This finding is typical for all cases studied.

The excretion of the steroids at later periods was followed in most patients, now up to about two years after the operation, and has in all cases remained at the same low level, 0–2 mg. per day, while the patients were off cortisone.

The changes in *the adrenaline test* (0·3 mg. of adrenaline subcutaneously) are shown in Table VIII. The eosinopenic response to adrenaline was lacking from the second post-operative week on. The usual decrease in the number of circulating eosinophils after cortisone administration was present in these cases.

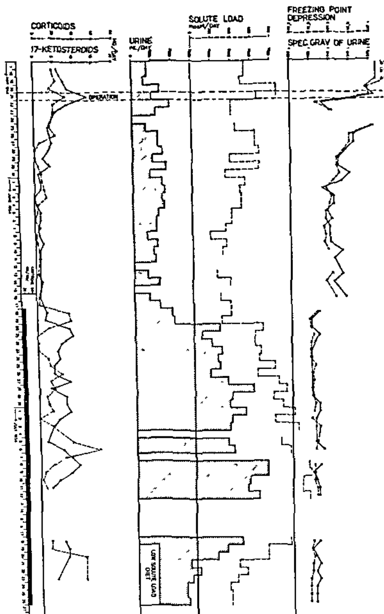


FIG. 1. Changes in the urinary excretion of corticoids and 17-ketosteroids, in the daily urine volume and solute load as well as in the specific gravity and osmolarity of the urine after hypophysectomy; the effect of cortisone administration on these changes.

The *sensitivity to insulin* was markedly increased in all patients tested, about ten, and a hypoglycæmia unresponsiveness, as seen in adrenal cortical insufficiency, was observed.

Table VIII

CHANGES IN THE ADRENALINE TEST AND THE URINARY EXCRETION OF 17-KETOSTEROIDS AFTER HYPOPHYSECTOMY IN METASTATIC MAMMARY CANCER

Case No	*Adrenaline Test*[1]				*17-Ketosteroids*[2]			
	Before Op.	*After operation*			*Before Op.*	*After operation*		
		2nd week	*3rd week*	*Later*		*2nd week*	*3rd week*	*Later*
1	66			0, 14, 18				0–2
2	+[3]	10	14	15	6–7	1	0	1–2
3	+	+	+	+	2–3			
4					4			
5		0		9				1
6	57	23, 28			3	2		0
7		0		0, 0, 27	5	1		0–1
8		4, 15	21	0, 10			1–2	1
9			3, 9	10	2		2	0
10		18, 40	2, 10		3			1
11	+	+	24		10		2	
12		12	32		2	1		0–1
13	82		12		8	1	1	0–1
14		0			3	1	1	
15	59	4			3	3		
16	48	0	0		3	1	1	
17	37	0	7		9	5	0	0–1
18	45		0, 3	10	3	0	1	0
19	48	13	6		4	1	1	
20	+	+	0		5	0	1	
21	+	+	60	0			6	0–1
22	70	23						
23		18	5		5	2		
24			15		2		0	
25		38	37, 62		8	7		
26					10		2	0
27			0, 15		4	1–2		
28		22	3		5	0–1	0	
29			19		6–10	1	0	0–1
30	+	11	+	+	14			1
Incomplete hypophysectomies								
A		0, 0						
B		0		40				4

[1] Decrease in eosinophils in % of initial value
[2] Urinary excretion in mg per day
[3] Too few eosinophils

This was especially pronounced in two diabetics after hypophysectomy, where the test had to be interrupted. When cortisone was given—25 mg. per day—there was still an increased sensitivity to insulin but less pronounced.

The changes in *water excretion* observed after hypophysectomy and the influence of cortisone thereon may be summarized as follows:

(1). The urine volume was increased in all patients during the first post-operative month. This polyuria was interrupted by a "normal interphase" with normal urine volumes showing the lowest volume around the seventh day (Fig. 2). The

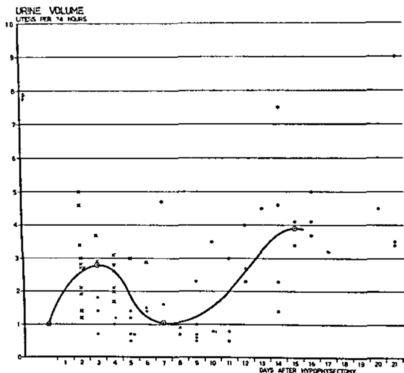


FIG. 2. The course of the diuresis after hypophysectomy in metastatic mammary cancer

× denotes the highest urine volume observed during the first week after operation,

● denotes the lowest volume observed during the first two weeks after operation;

○ denotes the highest volume observed after the oliguric phase.

administration of ACTH or cortisone post-operatively did not influence these changes in the diuresis.

(2). The level of diuresis during the second month was dependent on whether cortisone was given (Fig. 1). While

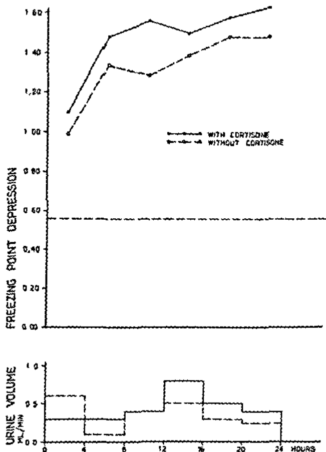


FIG. 3. A 24-hour thirst test in a hypophysectomized patient 14 months after the operation.

off cortisone the urine volumes were generally lower than two litres per day; when cortisone was given, usually 25 mg. per day, the daily urine volume increased markedly, in a few cases up to ten litres.

(3). This increase was parallel to an elevation of the daily urinary solute load as well as a lowering of the specific gravity of the urine from a level of 1·010—1·015 down to 1·005.

(4). The initial polyuria described above disappeared in most patients after three to six months, and the daily urine volume decreased to about 1–1½ litres with a specific gravity of about 1 012–1 015, while the patients were still on cortisone. If cortisone administration was discontinued the urine volume decreased somewhat further, and the specific gravity increased to 1 018–1·020. When a thirst test was performed a concentrated urine was obtained whether cortisone was given or not (Fig. 3).

(5). *In four patients the polyuria continued during the* whole period of observation. These cases have not been studied while off cortisone administration.

(6). During a water load the diluting ability of the kidney was normal only when cortisone was given. When cortisone was not given, the urinary specific gravity decreased but to a small degree, and the water excretion was delayed. This difference was observed during the immediate as well as the later post-operative periods.

The studies on the water excretion show: that the immediate post-operative polyuria and the normal interphase are *independent of cortisone or ACTH administration; that the* administration of cortisone is a prerequisite for the polyuria observed between the second and sixth month and for a normal response to a water load; and that the concentrating ability of the kidneys observed during the later periods is not influenced by cortisone. The changes in diuresis and in the response to a water load when cortisone was not given are consistent with the view that the amount of cortisone-like steroids was markedly decreased or absent in our patients after hypophysectomy.

To summarize· All tests used show that hypophysectomy induced an obvious adrenal cortical *insufficiency, which* remained for the whole time of observation.

Summary

A report is presented on the results obtained with hypophysectomy in advanced metastatic carcinoma of the breast and in severe diabetes mellitus with malignant vascular complications Special attention is given to the effect of hypophysectomy on the adrenal cortical function. The results obtained indicate that a permanent adrenal cortical insufficiency was obtained.

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DISCUSSION

WILKINS: What is your criterion of complete hypophysectomy? In seven cases that came to autopsy at Johns Hopkins that were supposed to have been complete, surprising amounts of hypophysial tissue were found

LUFT Our criterion is that the excretion of 17-ketosteroids and corticoids is almost 0, and there is a negative adrenaline test. In the beginning we just made a curettage of the sella, and some cases that were followed up had a normal response to adrenaline and the 17-ketosteroids rose again. In one of these we found a single layer of pituitary cells in one corner of the sella Since then we have cauterized the sella with formalin and acetic acid, and have had no case of incomplete hypophysectomy as far as we know.

HARRIS: These adrenaline tests are done after a long interval without cortisone?

LUFT: Yes.

QUERIDO: As a test of the completeness of hypophysectomy, what about the absence of gonadotrophins in the urine?

LUFT. We have no animal quarters in our department, and tests would have to be sent away. We haven't done this.

QUERIDO. Have you, Dr. Wilkins?

WILKINS: No, not routinely.

MACH. Have you observed any relation, Dr. Luft, between the age of your patients and the importance of the clinical signs of adrenal insufficiency, for instance the fact that hypoglycæmia is more important in younger people than in old?

LUFT: There is generally more trouble with complications in young patients. They get more swelling of the subcutaneous tissue, and seem

BAYLISS. Dr. Caughey of New Zealand is very interested in these cases of pituitary coma, and has observed patients whose symptoms have been attributed to hypoglycæmia, but who have normal blood

bouring structures in the hypothalamus?

SOFFER: *Do these patients tend to develop recurrences of these* episodes during the course of some months, or is this the only episode of this type they have?

LUFT They have had these episodes only once. We have made every effort after the operation to keep the blood sugar high, at about 500 mg. per cent during the first month. The patients seem to have very little, if any, ketone body formation

THORN: It seems to me that convulsions in a diabetic who had not had convulsions previously might very well be due to cerebral œdema. The low dosage of cortisone would probably condition that. Have you tried cortisone at the time of these reactions?

LUFT: Yes.

COPE: These comas are well known in hypopituitarism. They are usually quite independent of epileptic episodes. Sometimes you have in

a day or two. In one patient they are provoked by any infection, stress or by worry. In both there was reduction in frequency of attacks after putting them on maintenance cortisone, and I wonder if they would be largely eliminated by increasing the dose?

LUFT: Yes, I think so. If you increase the dose of cortisone from 5 to 10 mg., you increase the blood sugar and the urinary sugar. I am stressing hypoglycæmia because of the fatal case I just described.

BROWNE: In regard to the absence of virilism, do you get falling out of body hair after hypophysectomy?

LUFT: Yes.

BROWNE. And when you give the testosterone propionate in the male, does the normal body hair come back?

LUFT: With testosterone, thyroid and cortisone, yes

BROWNE: How soon does that happen?

eral weeks, and during

hyroid you give? You mentioned 50 mg.

LUFT: These patients are very sensitive to thyroid, of course. We gave them the equivalent of about 90 μg. of thyroxine.

PRADER. Did you notice that this sensitivity depends on the dose of cortisone you give?

LUFT: I don't know; we give all of them about the same dose of cortisone.

PRADER: And what about the serum electrolytes? There are reports on neurogenic hyper-electrolytæmia in the American literature, and we have observed the same condition in infants with hypothalamic damage.

LUFT: Yes, I have seen that in the literature. We have not noticed any disturbances of the electrolyte balance.

THORN: Would you enlarge on the fact that these patients show responsiveness to administered thyroid hormone? This is quite contrary to general clinical thought. You do have evidence that they respond well to this level of thyroid medication?

LUFT: The question is difficult to answer. I do not give these patients more thyroid than the dose I mentioned—they don't feel well when they get more. But their basal metabolism is not normalized by this dose level.

SPENCE. They are not so sensitive if you give them testosterone as well.

QUERIDO. I think this question of thyroid depends on whether their

to 3–4 μg.

LUFT. We studied PBI as well as the thyroid clearance and renal clearance of radioactive iodine in some of these patients. The PBI seems

to go down to low values after hypophysectomy. And all cases except one have a thyroid clearance of about 0. We do not see this immediately

BERGENSTAL: Our experience has been very similar to that of Dr. Wilkins. Our first five cases were done with a neurosurgical technique, using silver nitrate as cautery, and all resulted in varying degrees of diabetes insipidus. All of them died subsequently of cancer, and at postmortem all of them had minute amounts of pituitary left, in the dura, under the anterior clinoid—sufficient probably to cause this diabetes insipidus. Three of those patients had also had adrenalectomy

With Dr. Rasmussen, our neurosurgeon, and Dr. Paul Harper, working in our radioactivity laboratories, we devised a new technique using radioactive yttrium oxide beads. These 1 mm. beads on irradiation in the uranium pile for about three days develop intense beta activity having an activity of 1,000,000 r equivalents 1 mm. centre, dropping off to 50,000 r equivalents 3 mm. It therefore gives you a very intense area of activity without the possibility of gamma irradiation to destroy the hypothalamus and adjacent cranial nerves In the monkey we found that by placing one bead (about 0 8–1 2 mc) in the centre of the pituitary we could entirely destroy the monkey pituitary, only occasionally damaging the hypothalamus.

and cut. The dura is left intact. We use special spinal needles which have

We have done nine cases with this technique. Three have died of pulmonary embolus, and other medical reasons, cardiac failure, coronary occlusion, six have survived We have varied our technique, using 4–6 beads, and varying the positioning of the beads These cases have no diabetes insipidus after operation

LUFT Dr. Bergenstal mentioned growth hormone. I should like to

refer to a case reported by Pearson. In this case oophorectomy was performed initially for mammary cancer. The patient then got testosterone, and finally the adrenals were removed. However, the cancer
n removed, and the
ient was then given
f progression of the
tumour.

WILKINS Can Dr. Luft tell us anything about his experience of hypophysectomy in carcinoma of the prostate, in view of the possible role of luteotrophic hormone?

wi
coi
pa

one metastasis in TH8, and this one showed an increased density on X-ray. We have done two more cases only very recently, and the

was still growing. Five weeks after hypophysectomy the mass had shrunk markedly. These studies are being continued.

MACH: What about the state of the adrenal at autopsy, after operation?

LUFT. It is decreased in size. So far, we have not made any histological studies of the adrenal after hypophysectomy.

THORN: How effective is testosterone in preventing the hypophysectomy atrophy of the adrenal?

LUFT: I could not tell.

FORSHAM: It is apparently very successful in rats but has been shown to be unsuccessful in monkeys by E. Knobil and in mice by J. H. Leathem.

early.

THORN: Dr. Bergenstal, you said in your paper that you observe a normal insulin tolerance test on 50 mg. of cortisone.

BERGENSTAL: This was a half dose of insulin; I was afraid to use a whole dose.

LUFT: We used an insulin dose of 0·1 unit per kg. of body weight, given intravenously. With Professor von Euler we have been studying the catechol amines in the urine during insulin hypoglycæmia. Healthy

are only preliminary data. When we give the hypophysectomized patients 25 mg of cortisone per day we still find hypoglycæmia unresponsiveness to some extent.

VIRILIZING ADRENAL HYPERPLASIA: ITS TREATMENT WITH CORTISONE AND THE NATURE OF THE STEROID ABNORMALITIES*

LAWSON WILKINS, M.D., A. M. BONGIOVANNI, M.D., G. W. CLAYTON, M.D., M. M. GRUMBACH, M.D.,† and J. VAN WYK, M.D.,†

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THE methods of diagnosing the adrenogenital syndrome have been described previously (Wilkins, 1948, 1952). Female pseudohermaphroditism due to congenital adrenal hyperplasia must be distinguished from types of intersexuality unrelated to the adrenal. When the adrenogenital syndrome develops postnatally in females, it must be differentiated from virilism due to arrhenoblastoma or the Stein-Leventhal syndrome, premature pubarche or constitutional types of hirsutism. In males, macrogenitosomia præcox due to the adrenogenital syndrome must be differentiated from other types of sexual precocity. In both sexes virilizing adrenal hyperplasia can be distinguished from adrenal tumours by the response to cortisone (Wilkins, 1952; Gardner and Migeon, 1952; Venning *et al.*, 1952; Jailer, *et al.*, 1954).

The methods and the results of treating virilizing adrenal hyperplasia with cortisone have been reported from our clinic (Wilkins, 1952; Wilkins, *et al*, 1950*a* and *b*, 1951, 1952*a–c*; Wilkins and Cara ,1954; Crigler, Silverman and Wilkins, 1952)

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†Fellows of the National Foundation for Infantile Paralysis

A portion of this report has appeared in the Festschrift for Professor Freudenberg, published by S. Karger, Basel, Switzerland.

and confirmed by others (Prader, 1950, 1953; Shepard and Clausen, 1951; Jailer, *et al.*, 1952; Jailer, 1953). The present discussion will bring up to date our results with continuous therapy for periods as long as four years and will present studies which shed additional light on the abnormalities of steroid synthesis occurring in this disorder.

As shown in Table I, 53 patients (39 females and 14 males) with the adrenogenital syndrome due to adrenal hyperplasia

Table I

TOTAL CASES AND DIFFERENT TYPES OF VIRILIZING ADRENAL HYPERPLASIA AND NUMBER OF CASES TREATED AND UNTREATED WITH CORTISONE

VIRILIZING ADRENAL HYPERPLASIA

NUMBER AND TYPES OF CASES

	TOTAL	VIRILISM ONLY	NA-LOSING	HYPERTENSIVE
FEMALES	39	26	10	3
MALES	14	7	5	2
TOTAL	53	33	15	5

CASES TREATED AND UNTREATED

	TOTAL	TREATED c̄ CORTISONE		UNTREATED	
		ALIVE	DEAD	ALIVE	DEAD
FEMALES	39	29	1*	6**	3
MALES	14	12	0	1	1
TOTAL	53	42		11	
		* DEATH UNRELATED TO DISEASE		** 4 RAISED AS MALES	

have been seen. Of these, 33 had simple virilism. In 15 virilism was accompanied by the Na-losing syndrome, and in five by hypertension. Forty-two patients have been treated with cortisone. One died of an unrelated illness but the others are still under treatment. Of 11 patients not treated, four died of sodium loss. Table II shows the duration of therapy;

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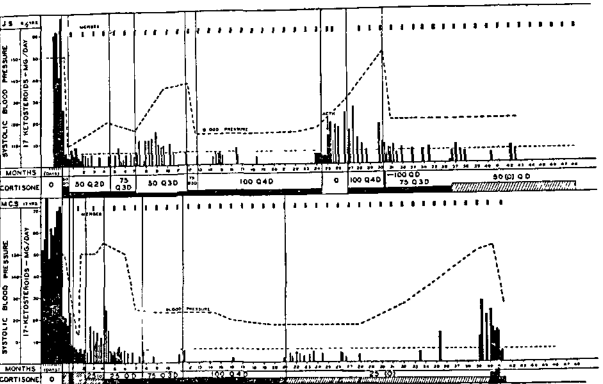


FIG. 1. Chart shows the method of regulating the dose of cortisone using the 17-KS output as a guide. Dotted line shows the average systolic blood pressure. Note that when the 17-KS rose above the minimum value hypertension recurred.

11 patients have received the drug continuously for over three years and 22 for more than two years.

Table II

DURATION OF CONTINUOUS TREATMENT WITH CORTISONE

DURATION OF TREATMENT

	TOTAL	FEMALES	MALES
OVER 4 YRS	2	2	0
3 - 4 YRS	9	7	2
2 - 3 YRS	11	5	6
1 - 2 YRS	11*	9*	2
6 MOS - 1 YR	8	7	1
< 6 MOS	1	0	1
	42*	30*	12

* ONE DEAD

[illegible] of Treatment

It has [illegible] initially by [illegible] daily in older children or adults and 25 mg. daily [illegible] In seven to ten days this causes the 17-ketosteroid output to fall to the minimal level which can be attained. This is usually 4–6 mg. per day in the older group and 0.5 to 1.5 mg. per day in young infants. The minimum dose of cortisone which is required to maintain adequate suppression should then be determined in each case on the basis of the ketosteroid output as shown in Fig. 1. Because of its slow, uniform rate of absorption only small doses of *intramuscular* cortisone are required which can be administered at intervals of three or four days. Intramuscular administration of cortisone is

Under two years of age, untreated infants show relatively little overgrowth in stature. In fact, those with uncorrected electrolyte defects may be malnourished and retarded in growth. In the absence of an abnormality of electrolytes, there is a gradual acceleration of epiphyseal development during this period; but in the untreated infant of the age of two years we have not seen the bone age advanced beyond the level of six years. Excessive somatic growth and premature epiphy-

Table IV

STATUS OF PATIENTS AT TIME THAT CORTISONE THERAPY WAS BEGUN

AGE YRS	NO. OF CASES TREATED			17-KS BEFORE Rx MG /DAY	OSSEOUS DEVELOPMENT YRS	DEGREE OF VIRILIZATION	
	TOTAL	FEMALE	MALE			FACIAL & BODY HAIR	PUBIC HAIR
OVER 20	4	4	0	25 - 60	ADULT	+++	+++
7 - 20	14	11	3	20 - 60	12 - ADULT	0 - +++	++
2 - 6	9	3	6	6 - 20	6 - 12	0	+ - ++
< 2	12	11	1	2 - 6	< 6	0	0
TOTAL	39	29	10				

seal ossification can be checked. The fact that the osseous development has not reached the point of impending epiphyseal fusion should make it possible for skeletal growth to continue long enough for patients of this group to attain reasonably normal adult stature, provided the dose of cortisone is properly regulated. Labial or pubic hair does not usually appear before the age of one-and-a-half to three years. Accordingly, when treatment is begun before this age, the growth of sexual hair and other evidences of progressive virilization are prevented. Adolescent feminine development does not begin in girls treated during this period.

When treatment is begun *between the ages of two and seven years*, statural growth and osseous development are frequently far advanced in respect to the chronological age. A patient of six years may have attained already the epiphyseal development of a twelve-year-old child, but since epiphyseal

preferable to *oral* which requires larger daily amounts divided into three or four doses. Table III shows that the average requirements vary from 8 mg. per day in infants to 25 mg. per day in adults when cortisone is given intramuscularly whereas from 15 to 75 mg. per day must be given when it is administered orally. It should be emphasized, however, that the optimum dose should be determined for each patient on the basis of the 17-KS output and the clinical response. In older patients a rise of 17-KS above 10 mg. a day is usually accompanied by a recurrence of hirsutism and

Table III

INITIAL AND MAINTENANCE DOSES OF INTRAMUSCULAR AND ORAL CORTISONE

AGE YRS	17-KETOSTEROIDS MG/DAY		DOSE OF CORTISONE - MG				
			INITIAL	MAINTENANCE			
	BEFORE Rx	UNDER Rx	I M	INTRAMUSCULAR	PER DAY	ORAL	PER DAY
OVER 6	20 - 80	4 - 6	100 Q D	75/3 0	= 25	25 x (2 OR 3)	= 50 - 75
2 - 6	8 - 20	2 - 4	50 Q D	50-75/3 0	= 16 - 25	12 5-25 x (2 OR 3)	= 25 - 50
UNDER 2	2 - 8	0 5 - 1 5	25 Q D	25-37 5/3 0	= 8 - 12	5-10 x (3 OR 4)	= 15 - 20

acne. In young infants the rates of growth and osseous development may be better guides to treatment than the precise output of 17-KS. Fortunately the doses of cortisone needed for the control of this disorder are approximately the physiological requirements as shown by others in adrenalectomised or Addisonian patients (Thorn, *et al.*, 1953). We have found no differences in the requirements of Compound F and cortisone, given either intramuscularly or orally.

Results of Treatment

The clinical effects observed under continuous cortisone therapy depend upon the level of somatic development and the degree of virilization which the patient has attained before treatment is instituted. Table IV shows the status of patients of different age groups whom we have treated.

ages of thirteen or fourteen years, the testes grew and developed rapidly. Biopsies after seven to nine months of treatment revealed active spermatogenesis and abundant Leydig cells. In the younger boys who did not show testicular development, the progress of virilization was arrested. The older

Table V

Effects of Cortisone on Testicular Development in Patients with Virilizing Adrenal Hyperplasia. Testes Drawn to Scale, Averaging Before Treatment 2·5 × 1·3 cm. In the 3 Older Boys Testes Increased to an Average of 4·5 × 2·7 cm. (Dots Indicate Testicular Biopsies.)

	Cortisone begun		17KS mg./day		Size of testes
	Chron. age	Bone age	Before	After	Months of treatment 0 3 6 9 12 15
S.B.	3 6/	10½	28	3.5	
W.J.	3 8/	9	10	2.5	24 MOS.
A.BR.	3 11/	9	8.5	2.3	
W.D.	5 9/	11	18	2	
M.D.	6 9/	13	40	4.5	
T.T.	6 10/	14	28	3	
D.BR.	8 9/	14	25	7	

boys continued to show progressive adolescent development due to the secretion of testicular androgens.

Four female pseudohermaphrodites were not treated until they were *between the ages of twenty-five and forty-nine years.* Three of these had had heavy beards for many years, requiring shaving once or twice a day. Although there has been some improvement, shaving is still necessary. One patient of

fusions have not occurred at this stage, further growth is still possible. Patients of this age usually have developed some sexual hair, but other evidences of virilization are not far advanced. The further progress of masculinization can be prevented by treatment. In some cases, one may observe after treatment the onset of early adolescent changes corresponding to the true sex of the patient, but the sexual development advances only slowly and gradually as in normal adolescence.

In patients treated *between the ages of seven and twenty years*, epiphyseal development is usually far advanced and fusions either have occurred or are imminent. There is little or no opportunity for further growth. Hirsutism already may be excessive and may have appeared on the trunk, face, and extremities. Treatment with cortisone checks the further progress of hirsutism, but considerable time may elapse before it becomes apparent that the hair is growing less rapidly and is becoming finer and lighter. When treatment is begun in girls whose osseous development is over twelve or thirteen years, there is usually rapid and spectacular development of female sex characteristics. The breasts begin to develop rapidly within a few weeks and menstruation may start after one or two months. The occurrence of ovulatory menstrual cycles has been verified by basal temperature curves and endometrial biopsies.

In males of this age, there is an equally rapid onset of adolescent sexual development (Wilkins and Cara, 1954). Table V shows the effects of cortisone therapy on the testes of seven boys of different ages. Before treatment, the testes were all small, measuring approximately $2 \cdot 5 \times 1 \cdot 2$ cm. Biopsies showed that the seminiferous tubules were either completely immature or that gametogenesis had not progressed beyond the stage of spermatocytes. Leydig cells were absent. In the boys of three to four years of age who had bone ages of nine to ten-and-a-half years, there was no growth or maturation of the testes under treatment but in those of six-and-three-quarters to eight-and-three-quarters years with bone

checked the advanced epiphyseal development which threatened to cause fusions, but at the same time permitted a normal rate of growth.

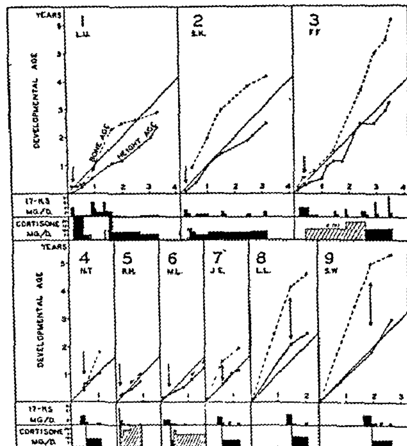


FIG. 2. Developmental charts of patients with virilizing hyperplasia treated with cortisone in early infancy. ——— indicates "height age", — — — "bone age".

In Case 1, excessive cortisone (25 mg /d.) given at first caused marked retardation of growth and osseous development and excessive growth occurred when treatment was omitted. In Case 3, failure to give adequate treatment permitted rise of 17-KS and acceleration of growth and development. In the other cases reasonably normal patterns of growth and development were attained.

forty-nine years who had marked masculine baldness before treatment, developed a new growth of gray hair on her scalp. In spite of their relatively advanced age, all the patients of this group showed rapid female development and the establishment of regular menstrual periods. We know of pregnancies occurring in two women treated in other clinics.

Effect of Cortisone on Somatic Growth and Development

In order to prevent excessive growth and osseous development with premature fusion of the epiphyses before adult height is attained, it is necessary to begin treatment early and to give sufficient cortisone to suppress the androgenic hyperactivity of the adrenals. On the other hand, if cortisone is given in amounts greater than the physiological requirements, protein anabolism, growth and osseous development are inhibited as they are in Cushing's syndrome. It is important to follow the rates of growth and osseous development as well as the output of 17-ketosteroids in determining the maintenance dose of cortisone. The results in some of our patients are shown in Figs. 2 and 3. In case 1, 25 mg. of intramuscular cortisone daily were given between the second and sixth month to a young infant with the Na-losing syndrome to study the effects on the electrolytes. This inhibited normal growth and osseous development and caused symptoms of Cushing's syndrome. The omission of therapy led to a rise of 17-ketosteroids and excessive growth and development. Subsequently, maintenance with doses of 6–8 mg. of intramuscular cortisone per day resulted in relatively normal growth. Case 2 showed a fairly normal developmental pattern, while Case 3 showed that failure to maintain adequate adrenal suppression (due to failure of the mother to administer cortisone as directed) resulted in exceedingly rapid epiphyseal development and growth. In Cases 4–9 reasonably satisfactory development patterns have been maintained. In Cases 10–11 the administration of cortisone to children six years of age

Our plan has been to carry out treatment in the following order.

1. Administration of 4–6 g. NaCl daily—orally or parenterally.
2. Administration of cortisone and adjustment of dosage to maintain the proper suppression of the 17-ketosteroids.
3. Determination of the daily intramuscular dose of DCA required to maintain normal serum electrolytes. Following this, the calculated number of DCA pellets are implanted and the intake of NaCl is reduced if this becomes indicated.

Table VI shows the treatment being used in ten patients with this disorder. Some of these infants were admitted to

Table VI

MAINTENANCE DOSES OF DCA, NACL AND CORTISONE USED IN CASES OF ADRENAL HYPERPLASIA OF THE NA-LOSING TYPE

TREATMENT OF Na-LOSING TYPE OF ADRENAL HYPERPLASIA

NAME	SEX	AGE		MAINTENANCE TREATMENT			
				NaCl	Cortisone	DCA	
		When Treated Mos.	At Present Mos.	Gm./day	Mg./day	125 mg. Pellets	Date Implanted
T.D	F	1	11	4	8.3 (i.m.)	5	9/53
R.K	M	2	14	3	20 (o)	3	7/53
G K.	F	2	9	2	8 3 (i.m.)	2	12/53
L U	F	2-1/2	49	3	8.3 (i.m.)	4	1/52
B K.	F	2-1/2	43	3	9 4 (i m.)	3	3/52
M L.	F	3	23	3	15 (o)	3	12/51
R A.	M	4	7	4	8 3 (i m.)	5	4/54
F F.	F	4-1/2	45	4	12.5 (i m.)	0	-
J A.	F	9-1/2	19	3	8.3 (i m.)	2	7/53
R B	M	42	72	5	75 (o)	3	11/49

Treatment of Congenital Adrenal Hyperplasia Combined with Defective Electrolyte Regulation

The treatment of infants with congenital adrenal hyperplasia who have a defect of electrolyte regulation presents a

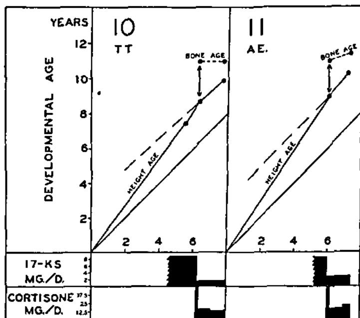


Fig. 3. Developmental curves of two children with adrenal hyperplasia treated with cortisone at the age of six years. Before treatment, growth and osseous development were markedly advanced. Cortisone caused slowing of growth to normal rates while little advance in osseous development occurred. This should delay epiphyseal fusion and permit the attainment of more nearly normal adult height.

difficult problem. Evidence has been presented by Crigler, Silverman and Wilkins (1952) that the disorder is not due merely to deficiency of Na-retaining hormone, but that the abnormal pattern of steroids secreted tends to promote Na loss. Cortisone is of benefit largely because it suppresses the abnormal secretion of steroids and prevents Na-losing crises. In most cases, treatment with desoxycorticosterone in addition to cortisone is indicated.

given 25 mg. orally. With a rise of ketosteroids hypertension recurred.

Table VII

Effects of Cortisone on Hypertension in Patients with Adrenal Hyperplasia

EFFECTS OF CORTISONE ON HYPERTENSION IN PATIENTS WITH ADRENAL HYPERPLASIA					
NAME	SEX*	TREATMENT BEGUN		BLOOD PRESSURE	
		Age (yrs.)	*Date*	*Before Treatment*	*At Present*
J.J.	F	25	8/51	160/110	130/90
J.S.	F	16	6/50	155/105	115/80
M.C.S.	F	16-1/4	11/50	155/110	120/80
W.J.	M	3-1/2	12/50	160/105	110/70
J.F.	M	3-1/2	10/53	136/80	100/60

Abnormality of Steroid Synthesis in Virilizing Adrenal Hyperplasia

It has been postulated by a number of workers (Bartter, *et al.*, 1951; Jailer, *et al.*, 1952; Wilkins, *et al.*, 1952*b*; Kelley, *et al.*, 1952) that in virilizing adrenal hyperplasia there is impairment of the ability of the adrenal cortices to synthesize gluconeogenic hormone (Compound F) from its precursors. That the impairment is probably only partial is suggested by the fact that only one patient has been described as showing clinical manifestations of deficiency such as hypoglycæmia (White and Sutton, 1951). Kelley, Ely and Raile (1952) reported low-normal levels of fasting blood glucose but paradoxically these levels were even lower on cortisone therapy. Kelley, Ely and Raile (1952), Bongiovanni *et al* (1954) and others have generally found the circulating levels of 17-hydroxycorticoids to be lower than normal in this syndrome. Mason (1951) and Kelley *et al* (1952) have reported an inadequate eosinopenic response to ACTH in this disorder, but it appears from the data of the latter that with larger doses of ACTH a significant eosinopenia sometimes occurred.

the hospital in a state of severe dehydration and collapse, in spite of the fact that they had been treated vigorously with NaCl, and in some cases with DCA. Others had been maintained in fairly good condition by this type of treatment, but they had failed to gain weight and still had abnormal serum electrolytes. After the institution of cortisone therapy, it was much less difficult to maintain electrolyte balance and smaller doses of DCA and NaCl were required. However, it seemed advisable to implant DCA pellets in all of our patients except one (F.F.). The number of 125-mg. pellets varied from two to five, depending upon the severity of the electrolyte disorder. These patients have remained in excellent condition and are growing and developing well. They have had the usual childhood infections without complications or tendency to lose sodium.

It is of interest that in three cases (S.B., L.U., and S.K.), long periods have now elapsed since the implantation of DCA pellets without renewal becoming necessary. Apparently, there is some tendency under the combined treatment to the recovery of the electrolyte-regulating function. We prefer the implantation of pellets to the intramuscular or sublingual administration of DCA not only because of its convenience but also because it leads to a very gradual withdrawal of therapy as the pellets absorb.

Effects of Cortisone on Hypertension Occurring in Cases of Adrenal Hyperplasia

The correction of hypertension by cortisone in three patients with virilizing adrenal hyperplasia has been reported previously (Wilkins, *et al.*, 1952*c*) and a similar case was published by Shepard and Clausen (1951). The results in five patients now under treatment are shown in Table VII. In one of these patients (J.S.) when cortisone therapy was omitted for three months during the summer of 1952 there was a recurrence of the hypertension with a concomitant rise of the urinary 17-ketosteroids as shown in Fig. 1. Her sister (M.C.S.) gradually escaped from cortisone suppression when

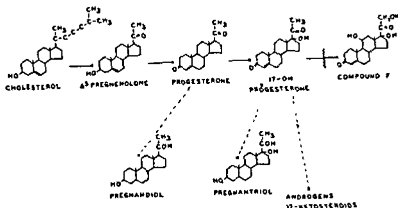


FIG. 4. Pathways of synthesis of adrenal steroids illustrating possible block in the adrenogenital syndrome.

Table VIII

AMOUNTS OF URINARY PREGNANETRIOL AND 17-KETOSTEROIDS EXCRETED BY PATIENTS WITH VIRILIZING ADRENAL HYPERPLASIA BEFORE AND AFTER CORTISONE TREATMENT

NAME	SEX	AGE YRS.	17-KS		PREGNANETRIOL	
			UNTREATED	c̄ CORTISONE	UNTREATED	c̄ CORTISONE
L S	F	34	46 ACTH ↓ 75		39 ACTH ↓ 61	
E W	F	30	31	4	44	0
D.W	F	16	33	21	37	12 (partial suppression)
L G	M	14	24 ACTH ↓ 29	2 2	45 * ACTH ↓ 233 *	0 *
F D	F	12	35	7	27	8 (partial suppression)
T D	M	12	19		14 *	0.2 *
D D	F	10	19	5	28	8 (partial suppression)
B B	F	10	31	6	34	0
B B	M	7	13	5	30 *	1 6 *
A B	M	6	6	1.7	16 *	0 4 *
F F	F	3	11	2	21	1 2
B W	F	2	4 1 ACTH ↓ 6.0	2	4 9 ACTH ↓ 10.5	0
L L	F	1-3/4	3 5		4 6	
B T	F	6 mo*	1 9		4 8	
S N	F	4 mo*	2 5 1.9	0 7	3 8 3 0	0
T C	F	1 mo	4 5		5 4	

* Spectroscopic measurement includes pregnanediol

	17-KS	Pregnanetriol
Normal adults	8-21	0.2-1 6
Normal children		0 -0 5

Although Kelley reported a failure of the circulating corticoic to rise following the administration of ACTH to individua with the adrenogenital syndrome, Bongiovanni detected significant rise in two of nine patients studied. The measur ment of urinary corticoids by various authors using sever methods has revealed levels which are normal or occasionall greater than normal. The latter is probably attributable t the lack of specificity of currently available techniques. I our laboratories, the quantities of corticoids in the urine c patients, measured after preliminary chromatography accord ing to the method of Nelson, have failed to indicate a deficienc of these substances by the application of reducing method (blue tetrazolium and phosphomolybdic acid), phenylhydra zine, and formaldehydogenic techniques.

From the findings just discussed it must be concluded tha the impairment of synthesis of 11-oxy-corticoids is relativ rather than absolute. A relative deficiency of gluconeogeni steroids would lead to increased secretion of ACTH, whicl would cause hyperplasia of the adrenals and might bring u the production of gluconeogenic hormone to physiologica requirements. Sydnor, *et al.* (1953) have demonstratec increased ACTH in the blood of patients with virilizing adrenal hyperplasia.

Jailer (1953) advanced the hypothesis that there is a block in the conversion of 17-hydroxyprogesterone into 17-hydroxy-corticosterone (Compound F) and that this precursor may be metabolized along abnormal pathways. This hypothesis has been substantiated by the finding of Bongiovanni, *et al.* (1953, 1954) that patients with adrenogenital syndrome excrete large amounts of pregnanetriol which probably can be derived only from 17-hydroxyprogesterone (see Fig. 4). The amounts of pregnanetriol found in our cases of virilizing adrenal hyper-plasia are shown in Table VIII and varied from 14 to 45 mg. per day in patients over ten years and from 3 to 21 mg. per day in infants of three years or less. Pregnanetriol was increased by the administration of ACTH and practically disappeared when the adrenal activity was suppressed adequately

Table IX

COMPARISON OF URINARY 17-KETOSTEROIDS AS DETERMINED BY PINCUS AND ZIMMERMANN REACTIONS. A LOW P/Z RATIO INDICATES LARGE AMOUNTS OF 11-OXY-17-KETOSTEROIDS

NORMAL ADULTS	17-KETOSTEROIDS (mg./24 hr.)		
	Pincus	Zimmermann	P/Z
M	7.0	9.5	0.74
	10.8	9.1	1.2
	7.6	6.8	1.1
F	3.2	3.9	0.82
	6.1	6.1	1.0
	2.8	3.0	0.93
	2.3	2.4	0.96
	4.1	5.0	0.82
	6.9	6.9	1.0
		Mean	0.95 SE ± 0.137
ADRENAL HYPERPLASIA UNTREATED			
SW	2.7	6.0	0.45
TD	0.7	1.5	0.47
PD	14.2	40.7	0.35
DD	10.0	26.6	0.38
SB	15.5	21.5	0.72
	13.0	24.2	0.54
LB	25.4	55.0	0.46
	35.0	60.0	0.58
NW	29.2	47.0	0.62
	36.0	54.3	0.66
SN	22.1	33.3	0.66
		Mean	0.54 SE ± 0.118
			P = less than 0.01

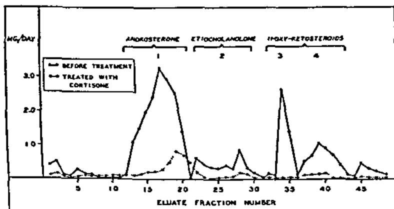


FIG. 5. Column fractionation of urinary 17-ketosteroids of a patient with virilizing adrenal hyperplasia before and after treatment with cortisone. The 3β-hydroxyketosteroids were removed previously with digitonin. The fractions were eluted with increasing concentrations of ethanol in benzene.

with cortisone. In normal adults the daily excretion of pregnanetriol was never greater than 1·8 mg. and in children was less than 0·5 mg. and was not increased by ACTH. The finding of increased amounts of pregnanetriol is of considerable clinical diagnostic value, helping to confirm the diagnosis of adrenal hyperplasia in very young infants, who may excrete less than 2·0 or 2·5 mg. of 17-ketosteroids per day, and serving as a guide in determining when complete suppression of adrenal activity is attained with cortisone.

The diversion of 17-hydroxyprogesterone into other pathways of synthesis is not completely understood. It is obvious that excessive amounts of 17-ketosteroids are formed but their source is not known. Apparently oxygenation at C_{11} can occur since a relatively large proportion of the total neutral 17-ketosteroids are 11-oxy-17-ketosteroids. This is shown by the fact that the values obtained by the Zimmermann reaction are considerably greater than those with the Pincus reaction (see Table IX). As shown by Dingemanse, *et al.* (1952) the 11-oxy-ketosteroids react less vigorously with the Pincus than with the Zimmermann method so that a low $\frac{\text{Pincus}}{\text{Zimmermann}}$ ratio indicates increased amounts of 11-oxygenated compounds. The relatively large amounts of 11-oxy-17-ketosteroids in an untreated case of adrenal hyperplasia and their suppression by cortisone are shown by the column chromatograms in Fig. 5.

The cause of the androgenic manifestations is not entirely known. Jailer believed that they might be caused by 17-hydroxyprogesterone, which has been shown to have some androgenic activity. Bongiovanni, however, has been unable to find increased 17-OH-progesterone in the urine or blood. It would seem reasonable to attribute the androgenic biological effects to the steroids responsible for the increased production of 17-ketosteroids.

The cause of the Na loss which occurs in some of the infants with adrenal hyperplasia is not clear. Crigler, Silverman and Wilkins (1952) have advanced reasons for believing that this

correction with cortisone is shown in Fig. 6. There is relative impairment in the synthesis of gluconeogenetic hormones, and their precursors are diverted toward the excessive production of androgenic and œstrogenic substances. To compensate for the relative deficiency of Compound F, ACTH is increased. This causes adrenal hyperplasia and may partially

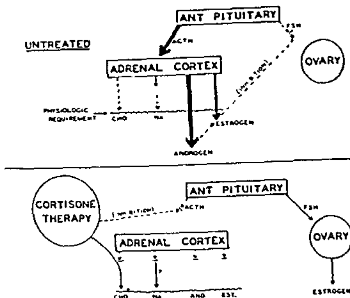


FIG. 6. Hypothesis concerning the mechanism of virilizing adrenal hyperplasia and its control with cortisone.

restore the production of Compound F to more physiological amounts. As it does not correct the abnormality of synthesis, increased amounts of androgen and œstrogen are produced. Œstrogen does not cause feminization because its peripheral effects are antagonized by excessive androgen. Androgen and œstrogen circulating
pituitary gonadotropl
do not secrete sex hor...
in physiological doses it substitutes for the production of

is not explained entirely by deficiency of Na-retaining hormone but that the general pattern of steroids secreted by the adrenal in these cases causes an active excretion of Na.

Finally, the cause of hypertension which occurs in other cases has not been explained. Proof is lacking that there are alterations in the electrolytes or in the blood volume. Until now studies on the pattern of steroids have shed no light on the problem.

Our knowledge concerning the steroids in virilizing adrenal hyperplasia is at present incomplete and may be summarized as follows:

1. The blood corticoids measured by the method of Nelson are usually low.
2. *The administration of ACTH generally does not produce* a rise in circulating corticoids although exceptions have been noted. It does however uniformly increase urinary pregnanetriol.
3. The urinary corticoids are usually not abnormally low but this may be due to limitations of current methods.
4. The total urinary neutral 17-ketosteroids are greatly increased. The effects of ACTH are variable but generally its administration causes relatively less increase than in normal individuals.
5. The ketosteroids oxygenated at C_{11} are relatively more increased than the non-oxygenated ones and almost disappear when cortisone is administered.
6. Pregnanetriol is greatly increased in the urine. It is increased by ACTH or 17-hydroxyprogesterone and practically disappears on the administration of cortisone.
7. Pregnanediol is not increased (Bongiovanni, 1954).
8. Œstrogens are greatly increased and decrease on the administration of cortisone. The relative proportion of œstrone, œstradiol and œstriol are not altered (Migeon, 1953).

The hypothesis which we have offered to explain the clinical manifestations of virilizing adrenal hyperplasia and their

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DISCUSSION

THORN: I understand you studied three age groups—under two, two to

dose; additional hormone hence would not be expected to induce a decrease in 17-ketosteroids

on that. The doses quoted by Dr. Thorn are those used for initial suppression, following which the 17-ketosteroid output is readily controlled on much smaller doses intramuscularly, as noted above. It

cortisone itself contributes only metabolites of this nature. Furthermore,

THORN: The fact remains that with the marked differences in size of the patients one should obtain about the same hormone concentration

i.e., 0·5–1·5, 2–4, and 4–6

Compound F and the entire pituitary-adrenal mechanisı put at rest. Since the adrenal no longer secretes exces sex hormones, the secretion of gonadotrophins by the pituit is released. The ovaries or testes are activated and nor development ensues along the pattern of the patient's sex, provided the proper level of general somatic matu has been attained. This level seems to be that which cor ponds to a bone age of approximately twelve or thirt years.

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steroids and the pregnanetriol, then that might make a difference. After they are inhibited you don't get much 17-ketosteroid when you come [illegible] of ACTH which is a very queer thing.

THORN: The point is, are you inhibiting the pituitary, or is the cortisone administration *changing the metabolism of the adrenal gland?*

suggests that pituitary suppression is a factor. However, the excretion of pregnanetriol almost always rose at once. This brings up an important point other than the comments—namely the intermediary metabolism of the C_{21} corticoids in this disease before and after therapy. [illegible] but [illegible] 17- [illegible] even

FORSHAM: You could test your theory by putting a patient on a constant infusion of ACTH for four to five days, which would give you a constant excretion of ketosteroids, and then start giving cortisone and see whether the ACTH effect could be suppressed. That would settle the question about the peripheral action of cortisone.

QUERIDO: Hasn't that been done by Bartter? And I have done it

on administering 60 mg. ACTH per day and the same dosage of cortisone, her ketosteroids were maintained at the pre-treatment value.

THORN: So you were able to bring the ketosteroids back with ACTH itself? Then it must be the method of administration and dosage.

WILKINS: Do you have any theory of the mechanism of salt loss in these patients, Dr. Thorn?

THORN: I'd be interested whether the renal tubular cells would show any anatomical abnormality from this adrenal androgen, corresponding to the effect of testosterone on the fifth segment of the proximal tubule.

WILKINS: We have tried administering testosterone in certain cases

BROWNE: On the other hand, one has to remember that in the normal

that have been mild salt losers and now are grown, and I believe Dr. Prader has such a case.

BAYLISS I asked because of this idea of a specific salt-losing hormone.

first case that we described in 1940, the reticular zone seemed to have overgrown everything and there was no glomerulosa present Other cases have died, and we have noticed at autopsy that they were also lacking in glomerulosa I pointed out that there was hyperplasia of the

ittle hesitant

. or the DCA implant runs out and the patient has been on maintenance cortisone for some time, the salt-losing syndrome doesn't return

WILKINS· You think that the zone may have recovered?

BAYLISS: Yes. Because ACTH secretion is depressed by the cortisone, the androgen-secreting cells become less active and this allows the cells

excess as part of this syndrome, that that may be one reason why you don't see Cushing's syndrome develop with some of the fair-sized dose schedules which you employ. You have used 17-ketosteroids as the indicator of pituitary suppression of cortisone, but if your theory holds, why don't you get increased 17-ketosteroids in the urine when you give ACTH?

THORN: But if the cortisone altered the metabolism of the keto-

show a rise after ACTH or insulin. This is in contrast to normal individuals wh

followed

Labhart,

In nine patients an eight-hour i.v. ACTH infusion

carried out. The mean eosinophil drop was 31 per cent compared to

of the i v. test.

six years old. His mean 17-ketosteroid excretion was 64 mg. per day. He had azoospermia and markedly enlarged testicles, as Dr. Wilkins has described in a younger patient. We wondered if the intratesticular tissue was of adrenocortical origin as Dr. Wilkins believes or of interstitial cell tissue as Landing and Gold believe (*J. clin Endocrin.*,

ment and decreased again when cortisone was discontinued. I am inclined to regard this response as a proof of the adrenocortical origin of the intratesticular tissue.

ut unfortunately we

s looked like adreno-

, said that it also re-

as done some animal

We have also tried to treat these patients with a long-acting cortisone from CIBA, giving one injection every month (*Acta Endocrin.*, 1953, 14, 341.) The suppression of the 17-ketosteroids is satisfactory for two weeks after the injection. Later the 17-ketosteroids rise again slowly The clinical result is just as good as with oral or regular i m. cortisone. The total amount necessary per month is lower than with daily oral cortisone and about the same as with regular i m. cortisone every fourth day

FORSHAM What kind of ester was this long-acting cortisone?

PRADER We tried several The best was cortisone acetate with crystals ten times larger than in the regular cortisone acetate.

blocked with cortisone to see if it would have a salt-losing effect, but it had a salt-retaining effect as in normals.

FORSHAM. Instead of using testosterone, which does retain sodium, couldn't you give large amounts of pregnanetriol (which is quite soluble), and a small amount of cortisone, and see whether you get a salt loss?

WILKINS: I think there are a lot of interesting experiments to do with pregnanetriol. And of course I'm very interested to try the new halogenated steroids in salt-losing cases It is probable that pregnanetriol is

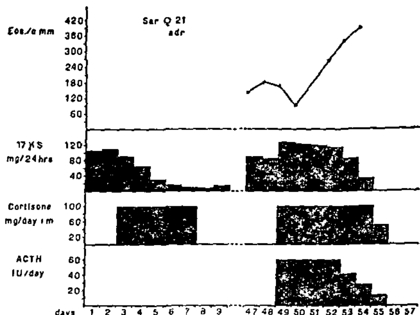


FIG. 1. (Quendo). (See text, p. 483.)

inactive; however we have administered 17-hydroxyprogesterone (its precursor), to patients and have not observed salt loss.

PRADER: We have treated 15 patients with cortisone for up to two

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In agreement with Dr. Wilkins and contrary to Kelley *et al.* (*J. clin. Endocrin.*, 1952, 12, 1140) we found regularly a normal blood sugar. The insulin tolerance test was done in eight patients, always giving a normal result.

Dr. Labhart and Dr. Froesch determined the plasma corticoids in some of our patients. They were normal or low and did not regularly

CUSHING'S SYNDROME*

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THIS report deals with 40 patients with classical Cushing's syndrome studied and followed by our group at the Mount Sinai Hospital over the past fifteen years. The series consists of 34 females and six males who varied in age from four months to 59 years, 64 per cent of the cases occurring between the ages of 20 and 40.

Signs and Symptoms

In Table I are listed the frequencies of the various signs and symptoms encountered in the group. It is of some importance to note that a very considerable majority of the patients manifested the characteristic moon-like facies, obesity, hypertension, some evidence of virilism, and disturbances in carbohydrate metabolism. In most instances the obesity involved the face, neck, trunk, and abdomen, the extremities remaining relatively thin. However, three patients had a diffuse obesity, and four individuals were quite thin. Eighty per cent of the female patients had varying degrees of virilism, and in Table II are listed the virilizing manifestations. In two-thirds of the females the evidences of virilism were relatively mild or moderate, but 15 per cent of the patients showed striking virilization. In only one-fifth of the group were the clinical manifestations those of pure Cushing's syndrome with no evidence of virilism. All six male patients showed some degree of feminization, such as a diminution in libido, a decrease in the size of the penis, some atrophy of the testes

*Aided by a grant from the United States Public Health Service.

WILKINS: I think that in the adult, with fused epiphyses, long-acting cortisone would be quite satisfactory from a clinical standpoint. But

that the 11-oxyketosteroids are increased. But 11-oxyketosteroids are of two types: the 11-oxyandrosterone type and the 11-oxyætiocholanolone. Now, if Dorfman's thesis is correct, it is a little doubtful that your 11-oxy titre would be 11-oxyætiocholanolone, because this is the derivative that would arise in largest portion from Compounds F and E; it is more likely to be 11-oxyandrosterone, which would come, not from C_{21} derivatives, but from C_{19} compounds—the adrenosterone type or 11β-hydroxy-Δ^4-androstenedione. So that if your methods are developed

present as stated. However, in view of Finkelstein's isolation of 11-ketopregnanetriol, it would seem that 11-hydroxylation can and does occur without 21-hydroxylation. Therefore it is not necessarily true that the 11-oxysteroids are derived only from Compounds E or F.

BAYLISS: I wondered, Dr. Wilkins, if you have encountered any

menopausal symptoms, but when we gave her cortisone they flared up and we had to put her on oestrogens

and prostate, or enlargement of the breasts. In only one male patient was feminization marked. As far as we could determine, the presence or absence of associated virilism in the female patients was not determined by any specific type of adrenal cortical tumour or any special location of the areas of adrenal cortical hyperplasia. Two of the six male patients had a carcinoma of the adrenal cortex; one had evidence of marked feminization, and in the other the feminizing features were meagre. Disturbances in the menstrual cycle, particularly amenorrhœa, occurred as the initial symptoms of the disease in one-third of the female patients, and obesity and virilism each occurred in one-third of the group as the first evidence of the illness.

Hypertension was present in slightly over 80 per cent of the patients, and in two-thirds of the group this was associated with cardiovascular-renal disease. In half there was electrocardiographic evidence of myocardial disease, in a third there was considerable cardiac enlargement, and two patients had overt congestive heart failure. In half the patients the retinal vessels were the seat of arteriolar narrowing alone or showed the additional presence of retinal exudates and hæmorrhages. Twenty of the 40 patients had some evidence of renal impairment, such as a significant albuminuria with an abnormal urinary sediment, and elevation of the blood urea nitrogen, a decrease in the concentrating ability of the kidney, or a diminution in the urinary excretion of phenolsulphonphthalein over a two-hour period. A hypercholesterolæmia varying from 300 to 665 mg. per cent was present in 78 per cent of the patients.

In Table III are listed the hæmoglobin, red and white blood cell counts and differential studies of the group. In only one patient was polycythæmia present, and this was of a mild degree. It is of interest to note that plethora, present in two-thirds of the patients, bore no relationship to either the hæmoglobin or red blood cell count level. Blood volume studies, employing either the Evans blue or the Congo red method, were performed in five members of this group and

Table I

SIGNS AND SYMPTOMS OF CUSHING'S SYNDROME (40 Cases)

	Number of Patients	*Per cent*
Moon Facies	36	90
Obesity: Central 32, Diffuse 3	35	88
Hypertension	32	80
Virilization	27*	79
Diabetes Mellitus**	30	75
Hæmorrhagic Manifestations	27	68
Amenorrhœa (non-menopausal)	23*	68
Plethora	25	63
Striæ	24	60
Œdema	24	60
Asthenia	23	58
Osteoporosis	22	55
Psychological Changes	17	43
Pathological Fractures	15	38
Telangiectasia	13	32
Cervical Fat Pad	12	30

*Of 34 females

**As manifested by symptoms, diabetic glucose tolerance curve, or elevated fasting blood sugar

Table II

VIRILIZING FEATURES (34 Females—Cushing's Syndrome)

	Number of Patients	*Per cent*
Menstruation		
Amenorrhœa (non-menopausal)	23	68
Menopausal	5	—
Normal menses	3	9
Menstrual irregularities	2	—
Pre-menarche	1	—
	34	
Alopecia	18	53
Acne	13	38
Facial Hair (Marked)	11	32
Masculine Pubic Hair	9	27
Enlarged Clitoris	8	24
Body Hair (Marked)	7	21
Lost or Diminished Libido	6	18
Masculine Voice	4	12
Atrophic Breasts	3	11

of the series, as well as the hæmorrhagic skin manifestations, are probably due to a thinning of the skin, the result of protein depletion, rather than to any intrinsic defect of the blood clotting mechanism or of the capillaries. The sedimentation rate was elevated in nine of 22 patients, and the increase in sedimentation rate bore no relationship to the nature of the underlying adrenal pathology.

Roentgenologically evident osteoporosis occurred in 22 of the 40 patients, 15 of whom had pathological fractures. The most common sites of such fractures were the ribs and spine, although one instance was encountered in the pelvis, one in the tibia, and one in the metatarsal bones. Apparently no relationship exists between the serum calcium and inorganic phosphorus levels and the presence or absence of osteoporosis. The serum calcium value was elevated in only one instance and normal in 32 patients, while the serum inorganic phosphorus was within the normal range in 23 instances. The serum alkaline phosphatase was increased in nine patients, seven of whom had overt osteoporosis and two of whom showed no roentgenological evidence of osteoporotic changes. A normal value for serum alkaline phosphatase was obtained in seven patients with osteoporosis and in eight patients without such bone changes. It would appear that an elevation of the serum alkaline phosphatase almost always indicates the presence of osteoporosis but a normal value by no means excludes the existence of such changes.

Calcium balance studies generally fail to reveal any significant increase in calcium excretion (Carter, *et al.*, 1954). However, the inherent defect in calcium and phosphorus metabolism can be brought out by the experimental administration of corticotrophin to patients with Cushing's syndrome. This procedure results in a markedly negative calcium and phosphorus balance, associated with a considerable increase in the fæcal and urinary excretion of these ions (Soffer, Gabrilove, and Jailer, 1950). The osteoporosis observed in patients with Cushing's syndrome is probably the result of at least two factors: (1) as postulated by Albright,

in only two was the blood volume slightly elevated. Half the patients had a mild increase in the total peripheral white blood cell count, and two-thirds had a mild leucocytosis. Almost 20 per cent of the remaining members of the group showed a definite lymphocytosis.

Table III

BLOOD COUNT AND DIFFERENTIAL STUDIES
(Cushing's Syndrome)

		Number of Cases
White Blood Cells/cu.mm.	4,000—10,000 10,000—15,000	23 (57%) 17 (43%)
Differential Count Polymorphonuclear Leucocytes . .	44—75% 76—89%	14 (37%) 24 (63%)
Lymphocytes . .	7—24% 25—56%	14 (37%) 7 (18%)
	Number of Cases	*Plethora*
Red Blood Cells million/cu.mm 3 5–4 0 . . . 4 1–5 5 . . . 5·6–6 7 .	 3 20 4	 2 17 2
Hæmoglobin grams/100 ml. 10–12 . . 12 1–15 . . 15 1–16 5 . .	 7 28 4	 4 19 2

Hæmorrhagic manifestations, such as ecchymoses, purpura, petechia, or easy bruisability, were present in 27 patients (68 per cent). Hæmatological studies, including coagulation time, bleeding time, prothrombin time, clot retraction, and blood platelets, were performed on 11 patients and found to be normal. A positive tourniquet test was present in four of 13 patients on whom this study was done. Both the plethora and violaceous striæ atrophica, the latter occurring in 60 per cent

Some years previously we pointed out the paradoxical effect observed following the injection of desoxycorticosterone acetate in patients with Cushing's syndrome (Soffer, *et al* 1944, 1949). Under the circumstances of the test, the injection of this steroid fraction in such patients following intravenous administration of a salt load often results in an increase in the urinary excretion of either sodium or chloride or both. The salt tolerance test was performed in 19 patients with Cushing's syndrome and a characteristic response was observed in 12 patients, or 63 per cent. In an additional three patients the response was equivocal and in four instances the results were entirely negative. An attempt was made to correlate the results obtained with the salt tolerance test and the serum sodium levels. In eight patients in whom a positive salt tolerance test was observed, the serum sodium level was within the normal range in five instances and elevated to 145 mEq./l. or more in three instances. Of the six patients who yielded equivocal or negative results with the salt tolerance test, five showed a considerable increase in the serum sodium levels. In general, it would appear that a positive salt tolerance test is less likely to be obtained in those patients with Cushing's syndrome in whom the serum sodium level is elevated.

In a further effort to define the nature of the response to the salt tolerance test, the influence of cortisone and desoxycorticosterone on the total body water, extracellular fluid, and sodium, chloride, and potassium balances was investigated in two patients with Cushing's syndrome, one of whom had previously demonstrated a positive test and the other a negative response. The results observed in the former patient were strikingly different from those obtained in normal individuals. Levitt and Bader (1951) have demonstrated that the administration of cortisone to normal individuals with a fixed sodium intake results in a considerable expansion of the extracellular compartment as measured by inulin. The increase in the extracellular compartment reaches a peak within eight to ten days and is then followed by a

the depletion of the protein material of the bony matrix *results in a decrease in the deposition of calcium for the formation of new bone*; and (2) a prolonged slight increase in the fæcal and urinary excretion of calcium and phosphorus.

Carbohydrate tolerance studies revealed that 79 per cent of the patients had a diabetic glucose tolerance curve, but only 27 per cent had an elevation of the fasting blood sugar level, generally associated with a glycosuria of varying degree. Two patients had a persistently flat glucose tolerance curve. Where the hyperglycæmia and glycosuria were severe, comparatively large amounts of insulin and rigid dietary restriction were required for even modest control, but no alarming degree of ketosis was ever encountered.

Blood Electrolyte Changes

In Table IV are listed the blood electrolyte changes observed in 33 patients. Of 93 determinations, 42 were abnormal and characterized essentially by an elevation of the serum

Table IV
SERUM ELECTROLYTES
(33 Cases—Cushing's Syndrome)

	Normal	*Elevated*	*Decreased*
Sodium	14	11	1
Chloride	17	10	1
Potassium	9	5	2
CO_2 Combining Power	11	10	2

Normal Na 135—145 mEq/l
Cl 95—105 mEq/l
K 3 5—5 0 mEq/l
CO_2 22 4—29 9 mEq/l

sodium, chloride, and potassium levels. Only rarely did we encounter a decrease in the serum levels of the latter two ions. An increase in the blood CO_2 content and combining power was observed in 10 of 23 patients in whom this determination was made, and was generally not associated with a demonstrable hypochloræmia or hypokalæmia.

The normal values in our laboratory vary from 0·75 to 2·0 mg. per twenty-four-hour urine volume. In the eight patients with increased urinary excretion of these fractions, the values

Table V
Excretion of Urinary Steroids
mg. per 24-hour volume
(25 Cases—Cushing's Syndrome)

	Neutral 17-Ketosteroids	*11-Oxygenated Steroids*
Carcinoma .	12·0	7·5
	18·3	2·4
	4·0*	
	254·0	0·0
	26·0	11·3
Adenoma . . .	4·2	–
	11·3	–
	20·9	–
	31·0	–
	7·2	–
Hyperplasia . .	11·4	2·3
	25·0	2·3
	23·4	7·2
	7·8	–
Normal Adrenal .	10·2	–
	8·2	–
	15·9	–
	29·5	–
	12·2	–
	26·0	–
Unproven Pathology .	9·1	–
	10·3	–
	20·4	4·1
	10·1	3·2
	2·4	–

*8-month-old female infant

varied from 3·2 to 11·3 mg. per twenty-four-hour volume, and in the remaining two patients the values were 2·4 and 2·3 mg per twenty-four-hour urine volume. In general, the values obtained in the instances with adrenal carcinoma were somewhat higher than those observed in the other

progressive decompression of the space despite the continued administration of the steroid hormone. In repeating these studies in normal individuals we obtained essentially similar results, employing sodium thiosulphate as a measure of the extracellular compartment and antipyrine for the determination of total body water. In normal individuals on a fixed daily salt intake of 3 5 g., we found that both cortisone and desoxycorticosterone resulted in gradual expansion of the extracellular compartment, which reached a peak increase of 20 to 35 per cent within eight to ten days. This increase in the extracellular space was not associated with any change in either the total body water or body weight and, therefore, probably represented a shift of electrolyte and fluid from other sources to the extracellular compartment. These studies were repeated in the patient with Cushing's syndrome who previously responded in a positive fashion to the salt tolerance test. It was found that following oral administration of cortisone there occurred a decrease in the extracellular compartment evident within three days after the beginning of the experiment and not associated with a previous increase in the extracellular fluid. There occurred no change in the total body water, and there was a slight but definite increase in the urinary excretion of sodium and chloride as contrasted to the control balance period. Identical results were subsequently obtained in this patient employing desoxycorticosterone acetate in place of cortisone. Similar studies conducted in the second patient with Cushing's syndrome who had not responded with a positive salt tolerance test yielded results identical in direction with those observed in normal individuals, although the changes were not quite so marked as seen in the latter group.

Urinary Neutral 17-Ketosteroids and 11-Oxygenated Steroids in Cushing's Syndrome

The daily urinary formaldehydogenic steroids were determined in 10 patients with Cushing's syndrome (Table V), and found to be elevated in eight and equivocal in two patients.

Forty-one serum cholesterol determinations were made in 30 patients, and a significant hypercholesterolæmia, with values ranging from 301 to 665 mg. per cent, was present in 78 per cent of the patients. Six of our 40 patients had a palpable thyroid, and 11 patients had definite mild clinical exophthalmos. The latter occurred entirely in the group of patients in whom the basal metabolic rate was elevated. It is of some interest to note that no members of the latter group had a tumour of the adrenal cortex.

Miscellaneous Clinical Observations

Six of the 40 patients of the group had roentgenologically demonstrable cholelithiasis and an additional three had nephrolithiasis. This constitutes a considerably higher percentage of calculi formation than is observed in a similar age and sex group of the normal population.

Varying degrees of mental and emotional disturbances occurred in about half the series. The manifestations of such disturbances included: depression, euphoria, paranoid trends, infantilism, marked emotional lability, and memory defects. Most of the members of this group were hostile, unco-operative, and often withdrawn. Two patients had serious and frightening overt psychotic episodes. Following successful treatment of the disease, the disturbing mental mainifestations gradually subsided, and the previously hostile or depressed patients became normally affable and co-operative.

Electroencephalographic studies were performed in ten patients, eight of whom showed perfectly normal tracings. The remaining two patients had abnormal tracings, one of which was interpreted as that of "diffuse cerebral dysfunction" and the other of "diencephalic dysfunction".

Visual field studies were conducted in 20 patients. In 19, the visual fields were well within the normal scale, but one patient showed a bilateral symmetrical constriction. Subsequent investigation revealed this patient to have a chromophobe adenoma of the pituitary.

groups, but there was a considerable degree of overlapping and the diagnosis of the specific type of adrenal pathology cannot be established on the basis of this determination.

The daily urinary neutral 17-ketosteroids were measured in 25 patients with Cushing's syndrome and in only ten of this group was the daily urinary excretion of these fractions in excess of 20 mg. Again, the highest values were in general obtained in the patients with adrenal cortical carcinoma, but considerable overlapping occurred among all the groups. A very marked increase in the daily urinary excretion of the neutral 17-ketosteroids is strong, but not incontrovertible, evidence in favour of an adrenal cortical carcinoma; but it must be emphasized that a normal daily urinary excretion of these fractions does not exclude the presence of an adrenal tumour nor does it serve to identify the precise nature of the adrenal pathology. A decrease in the urinary excretion of the neutral 17-ketosteroids in patients with classical manifestations of Cushing's syndrome has been reported to occur in the presence of a benign adrenal cortical tumour (Forbes and Albright, 1951). Such a finding was present in two of five patients with benign adrenal tumours in our group, but instances of low urinary neutral 17-ketosteroids were observed in isolated instances among other groups in our series. A significant percentage increase in the urinary excretion of dehydroisoandrosterone in association with a considerable increase in the total urinary excretion of the neutral 17-ketosteroids favours, but is not pathognomonic of, the presence of an adrenal cortical carcinoma.

Thyroid Function in Cushing's Syndrome

Forty-five basal metabolic rate determinations were made in 35 patients of our series. The basal metabolic rate varied from plus 15 to plus 48 per cent in 11 per cent of the group; from plus 15 to minus 15 per cent in 58 per cent of the patients; and from minus 15 to minus 36 per cent in the remaining 31 per cent. A normal or low basal metabolic rate, therefore, was found to be present in almost 90 per cent of the group.

satisfactorily to pituitary irradiation would suggest that an adrenal cortical tumour, either benign or malignant, was not the causative factor. In any event, of the proven operative or post mortem cases, the disease was due to a tumour of the adrenal cortex in slightly over half the patients; the incidence of benign tumour was approximately equal to that of carcinoma. In almost one-third of the patients the adrenal glands were normal in size, and in the remaining group the adrenals were hyperplastic. Post mortem studies in four patients with adrenal cortical carcinoma and in three patients with benign adrenal cortical tumour revealed the contralateral adrenal to be significantly atrophic in each instance (Table VII).

Table VII
ADRENAL AND OVARIAN PATHOLOGY
(8 Post Mortem Examinations—Cushing's Syndrome)

Patient	*Age*	*Adrenal Pathology*		*Ovarian Pathology*	*Duration of Amenorrhœa*	*Virilization*
		Right	*Left*			
M R	34	A	Ca	Normal	2 months	Moderate
A G	34	Aden	A	Small follicles A Corpus lutem	5 months	Moderate
G W	37	N	N	Fibrosis, occasional Corpora albicantia	Several months	Moderate
E S	37	A	Aden	No mature follicles No Corpora lutea	7 months	Moderate
V B	38	A	Aden	Atrophic, few follicular cysts	8 months	None
A R	37	Ca	A	Ova, Corpora atretica	17 months	Striking
M D	49	Ca	A	Fibrosis, Absent follicles	5 Years	Moderate
S L	63	A	Ca	Fibrosis, Bilateral cysts	16 Years	Moderate

A = Atrophic
Aden = Adenoma
Ca = Carcinoma
H = Hyperplasia
N = Normal

X-Ray Visualization of the Adrenals

Perirenal or retrorectal oxygen insufflation studies, with or without tomography, were performed in 27 patients with Cushing's syndrome, and intravenous pyelographic studies are available in 14 patients. In the group in whom insufflation X-ray visualization studies were done, 12 were patients

Pathology of the Adrenal Gland

In 33 of the 40 patients of our group, one or both adrenal glands were grossly and histologically examined either through operative removal or at post-mortem study (Table VI). Of these 33 patients, 18 were found to have an adrenal

Table VI
ADRENAL PATHOLOGY AND SEX INCIDENCE
(40 Cases—Cushing's Syndrome)

	Female	*Male*	*Total*
Pathology			
Normal	6	4	10
Hyperplasia	5	—	5
Adenoma	7	1	8
Carcinoma	9	1	10
	27	6	33
Unproven Pathology	7	0	7
	34	6	40

cortical tumour, of which ten were instances of carcinoma and eight were benign tumours. Of the remaining 15 patients, the adrenals in ten were normal in size grossly, each gland weighing from 5 to 8 grams. Histological studies of these glands, such as are conducted in a general hospital and not utilizing any very special staining techniques, failed to reveal any significant abnormalities. In the remaining five patients, the glands were definitely enlarged and hyperplastic. We could demonstrate no definite correlation between changes in width or structure of the zona reticularis with the degree of virilism which these patients with Cushing's syndrome manifested clinically.

In the remaining seven of the forty patients the nature of the adrenal pathology was never established, since these patients were neither operated upon nor did they succumb to the disease. The fact that all members of this group responded

satisfactorily to pituitary irradiation would suggest that an adrenal cortical tumour, either benign or malignant, was not the causative factor. In any event, of the proven operative or post mortem cases, the disease was due to a tumour of the adrenal cortex in slightly over half the patients; the incidence of benign tumour was approximately equal to that of carcinoma. In almost one-third of the patients the adrenal glands were normal in size, and in the remaining group the adrenals were hyperplastic. Post mortem studies in four patients with adrenal cortical carcinoma and in three patients with benign adrenal cortical tumour revealed the contralateral adrenal to be significantly atrophic in each instance (Table VII).

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Perirenal or retrorectal oxygen insufflation studies, with or without tomography, were performed in 27 patients with Cushing's syndrome, and intravenous pyelographic studies are available in 14 patients. In the group in whom insufflation X-ray visualization studies were done, 12 were patients

with a proven adrenal cortical tumour, and 15 were instances in whom no adrenal tumour was subsequently found on bilateral adrenal exploration. The intravenous pyelographic studies are reported only in patients with tumour. As can be seen in Table VIII, the correct pre-operative X-ray diagnosis

Table VIII
X-RAY STUDIES OF THE ADRENAL GLANDS
(27 Pathologically Proven Cases—Cushing's Syndrome)

	Perirenal and Retrorectal O_2 Insufflation Studies		
	Number of Cases	*Correct X-ray Diagnosis*	*Technically Inadequate*
Without Tumour	15	7	3
With Tumour	12	8	1
Total	27	15 (56%)	4 (15%)

	Intravenous Pyelogram Alone	
	Number of Cases	*Correct X-ray Diagnosis*
Adenoma	7	0
Carcinoma	7	4
Total	14	4 (29%)

was established almost twice as frequently with the insufflation technique as with the more orthodox intravenous pyelography. Of the 27 patients subjected to insufflation adrenal visualization, the correct diagnosis concerning the presence or absence of an adrenal tumour was established in 15 patients. The instance of diagnostic error was greater in the patients without tumour than in those with such a growth. In the former group, the correct diagnosis was established in seven of fifteen patients, and in the latter in eight of twelve patients. The presence of a suprarenal fat pad often served to convey the erroneous impression of the presence of a

tumour. Intravenous pyelographic studies were done in 14 patients in whom an adrenal tumour was subsequently found at operation. With this X-ray technique the presence of a tumour was correctly established pre-operatively in four instances.

The smallest tumour recognized before operation by perirenal or retrorectal insufflation weighed 6 grams, and the largest tumour missed with this technique weighed 100 grams. The smallest tumour recognized pre-operatively by intravenous pyelography weighed 30 grams and the largest tumour missed weighed 170 grams.

The Treatment of Cushing's Syndrome

The presence of an adrenal cortical tumour calls for prompt surgical removal of the growth. Prior to the advent of cortisone and corticotrophin, removal of a benign adrenal cortical tumour in patients with Cushing's syndrome was associated with a considerable immediate post-operative mortality. This was due to the fact that the contralateral adrenal was atrophic, so that following the removal of the tumour the patient, for all intents and purposes, was a bilaterally adrenalectomized individual until such time as the function of the contralateral atrophic adrenal approached normal. The post-operative mortality was somewhat less in patients with adrenal cortical carcinoma only because in most instances recognition of the disease occurred at a time when viable metastases were already present, which were capable of some adrenal cortical function.

Today, however, the patient with an adrenal cortical tumour may be successfully prepared for operation and safely carried through the hazardous post-operative period with the use of corticotrophin and cortisone The former is used for several days pre-operatively and for a short period post-operatively in an effort to stimulate the atrophic contralateral adrenal, while Compound E or F is administered on the morning of the operation and for such a period post-operatively as

with a proven adrenal cortical tumour, and 15 were instances in whom no adrenal tumour was subsequently found on bilateral adrenal exploration. The intravenous pyelographic studies are reported only in patients with tumour. As can be seen in Table VIII, the correct pre-operative X-ray diagnosis

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Table IX
COURSE AND TREATMENT
(33 Cases—Cushing's Syndrome)

	Without Tumour		Benign Tumour		Carcinoma	
	Number of Patients	*Number with Remission*	*Number of Patients*	*Number with Remission*	*Number of Patients*	*Number with Remission*
Pituitary Irradiation	6	6				
Pituitary Irradiation and Unilateral Adrenalectomy .	5	3				
Unilateral Adrenalectomy .	4	2	8	3	10	2
Total	15	11	8	3	10	2
Duration of Remission .		8 Months to 13 Years		6 Years 7 Years 10 Years		2 Years 7 Years

to tide the patient over the critical interval. The successful removal of a benign adrenal cortical tumour results in a cure of the disease. It is important to emphasize that adrenal cortical carcinoma is by no means an entirely hopeless illness. Two of our ten patients with adrenal cortical carcinoma are alive and completely well two years and seven years respectively after the removal of the adrenal tumour (Table IX). As the disease is recognized earlier and the correct pathological diagnosis more readily established, one may rightly expect an increase in the incidence of cure of adrenal carcinoma.

The question of treatment centres essentially around those patients with Cushing's syndrome in whom no adrenal cortical tumour is present: the instances of pituitary basophilism. One would not argue with the fact that total or almost total bilateral adrenalectomy in such patients would result in a very high incidence of remission of the syndrome. However, if it is possible to achieve an adequate lasting remission with more conservative and less hazardous measures, such procedures would be desirable. In Table IX are tabulated the results obtained in 15 patients with Cushing's syndrome in whom no adrenal cortical tumour was found at bilateral adrenal exploration. Six of this group developed a satisfactory remission following pituitary irradiation alone, in a dosage of 4500 r. delivered to the pituitary through six portals over a twenty-one-day period. Of five additional patients who failed to respond satisfactorily to irradiation therapy to the pituitary, subsequent unilateral adrenalectomy followed by another course of pituitary irradiation resulted in a satisfactory remission of the disease in three instances. The remaining four patients were subjected to unilateral adrenalectomy alone. Two of these patients developed a remission. Thus, of 15 patients treated with pituitary irradiation, unilateral adrenalectomy, or a combination of both procedures, remissions were obtained in 11 instances. In eight of the 11 instances the clinical remission obtained was complete, while in the remaining three patients the remission was partial but satisfactory. The partial remissions occurred

CUSHING'S SYNDROME

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My presentation differs from that of Dr. Soffer, in that I propose to deal with only a few cases and go into some detail regarding their progress.

Enlargement of Sella Turcica

In his abstract Dr Soffer mentioned the question of the enlargement of the sella turcica in Cushing's syndrome. It has been said that the sella is practically never enlarged in Cushing's syndrome. In two of our cases the sella turcica was enlarged. In one of these, which I am going to present later in more detail, there was at autopsy a curious pathological lesion which I had not heard of before, consisting of a cyst (possibly craniopharyngeal) above and posterior to the pituitary, with a basophilic adenoma at the right of the sella turcica which had eroded the sphenoid, pushing the right cavernous sinus laterally. It was not connected with the pituitary itself, which was beneath the cyst, and was perfectly normal in size and shape. The wall of this cyst was infiltrated with cells which were regarded by Dr. C. J. MacMillan, our pathologist, as basophilic, and as probably malignant. At autopsy the right anterior and posterior clinoids were eroded. On X-ray this sella was definitely enlarged, there was undercutting of the anterior clinoid, displacement and erosion of the posterior clinoid, and depression of the floor on the right side. (I am indebted to Dr. W. J. Shannon, Pathologist to the Cornwall General Hospital, Ontario, for this autopsy report.)

in two patients treated with pituitary irradiation and in one patient treated with unilateral adrenalectomy alone.

The duration of the remission of the disease has varied from eight months, in the instance of the most recent patient, to thirteen years. In view of the results thus obtained, pituitary irradiation or pituitary irradiation with unilateral adrenalectomy would appear to be reasonable procedures in the management of Cushing's syndrome without adrenal tumour. Only after failure with these techniques should one resort to total or subtotal bilateral adrenalectomy.

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[Discussion of this paper was postponed until after the paper by Prof. Browne—*Ed.*]

blood pressure and sugar tolerance became normal, he ceased to fracture his ribs, and his whole appearance changed. He has had no further treatment and he has remained normal since that time.

Renal Stones

In a case of a girl of twenty-one years, the first symptom of Cushing's syndrome was renal colic, and a renal stone was removed. I should like to know whether other people have seen renal stone as initial evidence of this syndrome. Our case developed typical signs and symptoms of Cushing's syndrome within months of the renal colic, and was treated with partial adrenalectomy on the left and complete adrenalectomy on the right. Another case, whom we saw at the age of twenty-four, had the onset of the disease nine years before admission, with obesity, irregular menses, hirsutism, florid face, striæ, etc. Renal stones had been detected in the left kidney five years previously. She came into hospital with a blood pressure of 230/150, renal failure with a P.S.P. of 25 per cent, marked impairment of sugar tolerance, renal stone, a serious pyelonephritis, and hydronephrosis. She died of a cerebral accident after removal of both adrenals and a left nephrectomy. Her early symptoms too were those of renal stone, which one might expect from the point of view of increased excretion of calcium secondary to osteoporosis, but which I have not seen described in the literature at the onset of Cushing's syndrome.

Excretion of Glucocorticoids, Chemical Corticoids, and 17-Ketosteroids

In the twenty-one-year-old girl with renal symptoms, the conjugated corticoids before operation were raised to 25 mg./twenty-four hours, compared with a normal running up to about 20 mg. in our laboratory. The glucocorticoids in this case were not raised (50–75 glycogen units). The free chemical corticoids, on the other hand, were definitely raised at 2·5–4·0 mg./twenty-four hours. We generally find an increase in

Testosterone Administration

In connection with Dr. Lawson Wilkins' remarks regarding the depression of 17-ketosteroids by cortisone, I should like to mention some data on the use of testosterone propionate to repress the excretion in the urine of glucocorticoid type of activity as determined biologically. In a case of Cushing's syndrome in which 25 mg. of testosterone propionate was administered daily, the urinary glucocorticoids fell practically to normal in three days. Accompanying this fall there was a normalization of the glucose tolerance curve. In a case of adrenocortical carcinoma with Cushing's syndrome in which the glucocorticoids were 400–700 glycogenic units, doses of up to 50 mg. testosterone propionate per day intramuscularly failed to cause any fall in glucocorticoids or alteration in the abnormal glucose tolerance test, which became more impaired, with rising fasting sugar, as time went on. There was, therefore, a parallelism in these two instances between capacity to depress the glucocorticoids, as measured by a decrease in the urinary excretion, and the alteration in carbohydrate tolerance.

Osteoporosis

I want to discuss the long-term progress of osteoporosis of the spine in another case of Cushing's syndrome. We first saw him in 1936. He was a classical Cushing's case, and his spine steadily got worse from 1936 to 1939. He had a mid-thoracic gibbous formation and collapse of some of his thoracic vertebræ. The vertebræ gradually became more and more decalcified. In the beginning of 1940 his pituitary was irradiated (2,500 r total dose), and he was given testosterone propionate, 25 mg. daily for eight months. He was in a Taylor brace at this time, and his gibbous formation disappeared. In December 1940 evidence of recalcification of the vertebræ was seen. This increased by 1943, and by 1945 calcification was practically normal. This recalcification also occurred in the pelvis. He had a complete arrest of his disease. His urinary corticoids returned completely to normal, his

Figure 1 shows the findings in a male patient with marked osteoporosis, impaired glucose tolerance, etc. He had an enlarged sella turcica (on X-ray) with a pituitary tumour at autopsy. The glucocorticoid values were very irregular, associated with our attempts to depress the corticoids with increasing doses of testosterone propionate (from 25–100 mg./24 hours); they tended to come down and then to escape again. At the 100 mg. testosterone dosage they were perhaps continuously repressed (Day 109–139). Obviously, as the dose of testosterone propionate was increased the 17-ketosteroids increased, but it is also clear that 17-ketosteroids coming from the adrenal itself were diminished (Days 148–150, compared with Days 1–8). A unilateral right adrenalectomy was done on Day 156, and it is interesting to note that the yield of ketosteroids from 100 mg. of testosterone propionate daily was very much less shortly after this operation than *before (Days 164–186 compared with Days 108–136); it* was only about half. The patient was still quite sick, and he may have had some liver disturbance, but there was no reason why he should have had further liver disturbance after the effects of the operation had passed off. He had less adrenal tissue after the operation, and I raise the question whether the amount and kind of adrenal cortical tissue present has anything to do with the metabolism of the administered testosterone propionate to 17-ketosteroids.

In Figs. 2, 3, 4 and 5 are demonstrated the findings in the case (a male aged forty-eight years) which has been most closely and continuously studied. (I am indebted for the history, the original diagnosis, and for the description of the final illness of this case to Dr. T. McEwen of Cornwall, Ontario, who referred him to me.) This is the patient with a cyst and basophilic adenoma, possibly malignant and separate from the pituitary, as mentioned on p. 505. In March 1950 his blood pressure was 110/70, in March 1953 150/80, in June 1953 155/100, in August 1953 180/105. At this time he developed marked œdema of the ankles, and on X-ray the heart was seen to be enlarged. He had a typical appearance

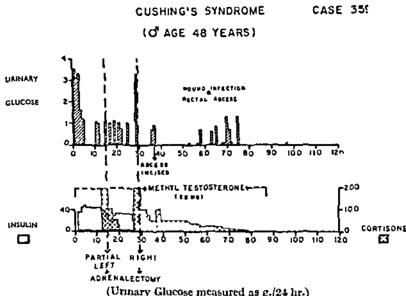
the glycogenic corticoids in the urine in Cushing's syndrome, but in this instance and in one other case they were perfectly normal. It is Dr. Venning's impression that tetrahydrocortisone and tetrahydro-F tend to interfere with the assay, so that it is possible to have very high chemical corticoids and normal glucocorticoids in certain cases (although this is not common), unless one chromatographically separates the interfering compounds from the biologically active substances. The nitrogen balance was at first slightly positive and then slightly negative.

This girl had two-thirds of the left adrenal removed on January 30, 1953, and was given cortisone because it was not known at the time whether she might have a tumour or not. The total neutral 17-ketosteroids were 17-22 mg. before adrenalectomy, but declined after the operation, and after the cortisone was stopped, to 10–12 mg /24 hours. The glucocorticoids were slightly raised under the influence of the cortisone but came down to about the same level as they were before the operation.

On November 16, 1953, a complete right adrenalectomy was performed, and she was again given cortisone. The 17-ketosteroids remained at about the same level (10–12 mg.) and so did the glucocorticoids, whether or not she was given 25 mg. of cortisone per day. The conjugated corticoids, which were still 25 mg./24 hours before the second operation, fell to 12–15 mg. after it

The left adrenal rest was shown to be perfectly capable of being markedly stimulated about twelve months after two-thirds of that adrenal had been removed—neither adrenal was found to be enlarged at the time of the operations. With ACTH the 17-ketosteroids rose, and there was a very marked rise of the glucocorticoids to 236 glycogen units; the free chemical corticoids increased from 1 75 to 3·78 mg./24 hours. Her glucose tolerance remained impaired, with fall of fasting sugar from 110 to 83 mg. Her menses returned after the first operation. She never showed any electrolyte abnormalities in the serum.

of Cushing's syndrome. In October 1953 his electrolytes were: Na 154, Cl 95·3, K 2·47, and CO_2 35·9 mEq./litre. His fasting sugar was 321 mg. per cent. He had polydipsia and polyuria and also a very marked glycosuria (Fig. 2), which is somewhat unusual in Cushing's syndrome, but he



(Urinary Glucose measured as g./24 hr.)

FIG. 2.

had it for a considerable period of time. The corticoid and 17-ketosteroid values are shown in Fig. 3. His glucocorticoids were not very markedly high; they were 80–100 glycogen units in October 1953. His conjugated and free chemical corticoids were high, 50 mg. and 2·5 mg respectively.

This man had a partial left adrenalectomy. The left adrenal weighed about 3·0 g. and four-fifths of it was removed. The right adrenal weighed 8 86 g. and was completely removed. He was given cortisone on both occasions, in the doses shown in Fig. 3. He developed a staphylococcyl

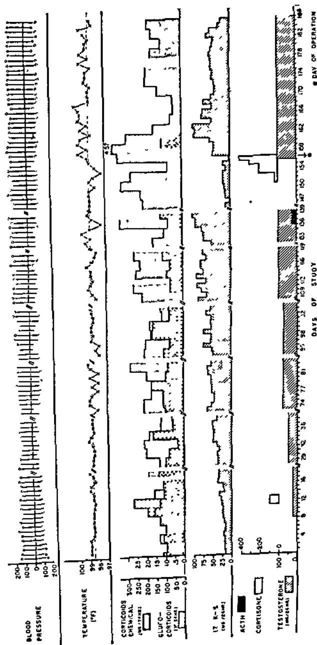

CASE-320
CUSHINGS SYNDROME
RM - AGE 26 YRS
BLOOD PRESSURE
TEMPERATURE (°F)
CORTICOIDS CHEMICAL
GLUCO-CORTICOIDS
17 K-S
ACTH
CORTISONE
TESTOSTERONE
DAYS OF STUDY
DAY OF OPERATION

FIG. 1.

infection in the left wound, and this became very much worse immediately after the second operation. He also developed an ischiorectal abscess and we wondered whether it was due to tracking of the original infection retroperitoneally, but the infecting organism was *Escherichia coli* and not *Staphylococcus aureus*. He was treated with various antibiotics and with Staphylococcus toxoid.

Prior to operation and between the first and second operations he received insulin 30–40 units daily (Fig. 2). The glycosuria diminished and remained constant in spite of cortisone. Shortly after the second adrenal was removed and after incision of the abscess in the left flank the glycosuria disappeared (Day 38) He received no insulin after Day 29, and was on 50 mg cortisone daily from Day 40–50. With the infection in the wound on the right side and the ischiorectal abscess (Days 52–75) there was a return of glycosuria but thereafter until death 114 days later there was no glycosuria in spite of marked terminal infection, when there was septicæmia, with abscesses in the ribs, kidney, etc. and purulent effusion in both pleural cavities, in the pericardium, and a large abscess in the area of the left adrenal wound. This absence of glycosuria was the more surprising in view of the fact that, as seen in Fig. 3, towards the end of the period in hospital the conjugated corticoids, free chemical corticoids, and glucocorticoids rose to or above their former level. The 17-ketosteroids did not quite reach their original level. At autopsy, five-and-a-half months after the first operation, the left adrenal remnant had regenerated from a fraction of a gram, say 0 5 g , to 16 g., and yet there was no recurrence of glycosuria. Figure 4 shows his glucose tolerance curves in August 1953, with a fasting sugar of 130 mg. per cent. In October 1953 the fasting blood sugar was once 321 mg. per cent, but no glucose tolerance curve was done at this time. In December, one month after the second operation, and in January 1954 (the curves are shown in Fig. 3) the fasting sugar fell but the curve was still impaired. Unfortunately no glucose tolerance curve was done in February when the

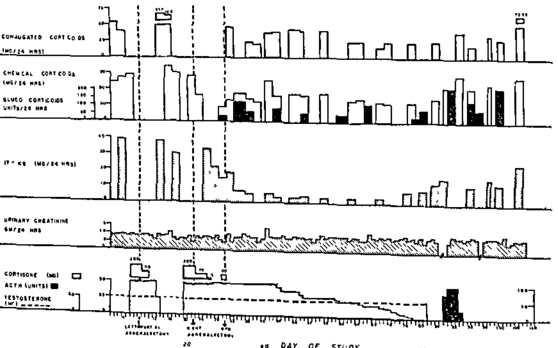

CASE 359
CUSHING'S SYNDROME
(♂ AGE 48 YEARS)
CONJUGATED CORTICOIDS
(MG/24 HRS)
CHEMICAL CORTICOIDS
(MG/24 HRS)
GLUCO CORTICOIDS
UNITS/24 HRS
17-KS (MG/24 HRS)
URINARY CREATININE
GM/24 HRS
CORTISONE (MG)
ACTH (UNITS)
TESTOSTERONE
RIGHT ADRENALECTOMY
20
40 DAY OF STUDY
80
100

Fig 8.

On inspection of Fig. 3 it will be seen that his conjugated corticoids *pre-operatively were very high*, as were the free chemical corticoids. The doses of cortisone were gradually reduced after the operation. The 17-ketosteroids fell to low levels. He was given 25 mg. of methyltestosterone throughout. When the methyltestosterone was stopped on Day 82 the corticoids began to rise (though I think that this was probably merely simultaneous, not causative). The chemical corticoids were in point of fact about the same at the end, just before he went home, as they were at the beginning. ACTH was administered from the 86–90th days (Fig. 3), and I will deal in detail later with the urinary chromatograms which were done at the time.

The patient was in positive nitrogen balance, as seen in Fig. 5, during the administration of the 25 mg. of methyltestosterone, in spite of all the cortisone, infection and fever. The dose of methyltestosterone was not large but apparently it was adequate to affect his nitrogen balance over a long period of time, as after it was stopped on the 82nd day (Fig. 5) the nitrogen balance became less positive. Administration of ACTH caused little change in nitrogen balance.

The chromatography of his urine during the last two days of ACTH administration is seen in Figs. 6 and 7. I should remind you that the left adrenal remnant had grown to an enormous size at the time of his death, two months later. The sodium-retaining effect is measured by the percentage retention of sodium in the urine of rats injected with the total neutral fraction. The free fraction is shown in Fig. 6 and the conjugated fraction after hydrolysis with glucuronidase in Fig. 7. In the free fraction (Fig. 6) elution of the preliminary chromatogram gives a mild degree of retention (36 minutes of urine). The middle chromatogram is of the Zaffaroni type. There is a smallish amount of F in the urine, and a somewhat large amount of E, which would contain the aldosterone fraction in this method of separation. The F fraction gives sodium excretion, the less polar substances perhaps slightly less sodium

corticoids were again raised, presumably due to the increasing regeneration of the left adrenal remnant.

I am unable to explain the absence of glycosuria and the lower blood sugar levels. They may be due to alteration of tissue response as a result of the infection (as Ingle has indicated in other forms of stress), to alterations in the

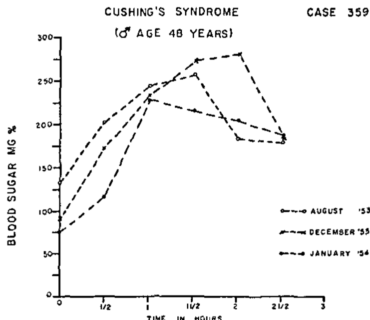


FIG. 4.

amount or type of substance affecting carbohydrate metabolism formed by the adrenal remnant regeneration, in spite of the higher glucocorticoid excretion in February—which may have been due to alteration of renal or liver function leading to a greater excretion of glucocorticoids though the amount produced by the adrenal was less. It should be noted that the total weight of the left adrenal was 16 g. at autopsy, whereas the weight of the two adrenals together originally was about 11·5 g.

excretion, the eluate here excluding the E fraction gives slight sodium excretion, and when the E fraction is separated out by the Bush method there is sodium retention in both fractions, with 36 minutes of urine.

In the conjugated fraction (Fig. 7), using the equivalent of 18 min. of urine throughout, there is more sodium retention

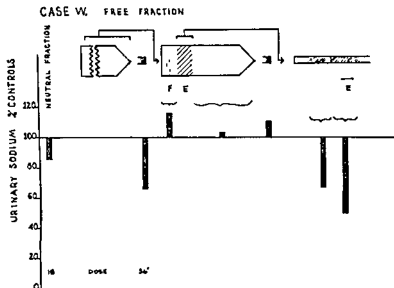
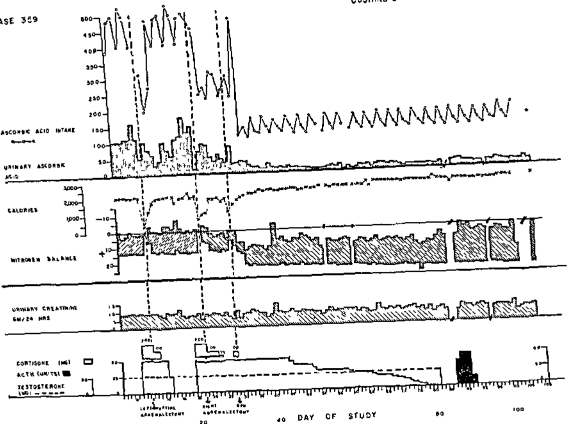


FIG. 6.

with the total neutral fraction. In the Zaffaroni separation, with the less polar substances there is a marked sodium excretion effect. In the Bush separation there is again sodium retention in both fractions as separated. Thus in fractionating the urine one finds opposing effects of the different fractions, and presumably the total neutral fraction effect on sodium retention will be a summation of these opposing effects. There are compounds apparently which produce very definite sodium excretion and they are not necessarily biologically active in other respects.



CUSHING'S SYNDROME, ♂ AGE 48 YEARS
CASE 359
ASCORBIC ACID INTAKE
URINARY ASCORBIC ACID
CALORIES
NITROGEN BALANCE
URINARY CREATININE GM/24 HRS
CORTISONE (MG)
ACTH (UNITS)
TESTOSTERONE (MG)
LEFT PARTIAL ADRENALECTOMY
RIGHT ADRENALECTOMY
DAY OF STUDY

FIG 5

incubation, the other is the addition of ^{14}C labelled progesterone or desoxycorticosterone. If they extract animal adrenal glands when they are *perfectly fresh they obtain no* steroids. On incubation different proportions of the following steroids appear on chromatographic separation: Compound B, one which they call X (which is 6-hydroxydesoxycorticosterone, and an unknown compound), Compound F and Compound E—and the Compound E spot will contain the aldosterone.

Various species are quite different from each other. Most of them contain a lot of B; the ox for example contains (these are proportions, not total amounts) B 50 to E1, F1 and X0·5. In the human being there is usually no B on straight incubation, but both in various animal species and in the human being, when one adds ^{14}C labelled progesterone or DCA, B is formed preferentially It is Dr. Heard's view that B is formed rapidly and is a transitional substance on the way to the formation possibly of F and E.

They have applied this technique to the left adrenal (3 g.) that was removed from R W. (Figs. 3, 4, 5), the patient most intensively studied. In this instance 1·07 g. was cut up and incubated, so that this is a *summation* of the action of all the adrenal tissue available and not simply of a small fraction of it. It was merely incubated, there was no radioactive substance added. The compounds were determined by ultraviolet absorption (Fig. 8). (They use E for Kendall's Compound E and E for electrocortin, so this peak is labelled EE). As shown in Fig. 8, the F peak is high. They would call these proportions 3 of F to 1 of E, no X (they have not found any X in the human being either on incubation or on addition of labelled progesterone or DCA), and B is also estimated as 0 because although there is a slight peak at the site where Compound B would be, they regard this peak as not being significant. This is the left adrenal gland (Figs. 3, 4) which was removed when the patient had the marked glycosuria and the marked electrolyte disturbance

The second gland (right) (Fig. 9) unfortunately is not

This patient (R.W.)'s carbohydrate tolerance improved. He continued, however, to have a high sodium throughout, and the sodium values rose again to 158-160 m.Eq./litre as the regeneration of the left adrenal occurred. He also had persistent œdema.

Dr. R. D. H. Heard, Dr. F. G. Perron and Dr. M. Cann of the Department of Biochemistry at McGill University have been

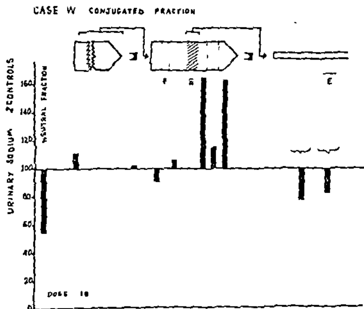


FIG. 7.

examining the effects of incubating adrenals and I am permitted to report their work here. Dr. Heard presented the preliminary findings in other respects at the Laurentian Hormone Conference of 1953, but he did not present the data on these patients. Their method is to cut the adrenal into "chunks"—Dr. Heard emphasizes that one cannot use thin slices, because the only thing which occurs in thin slices is the oxygenation at $C_{(11)}$ and no oxygenation or hydroxylation of the side-chain. They use two methods: one is simple

a good deal of E and very little F. The different proportion of F to E as compared to the first gland removed may be significant. The presence of B is due to the added DCA. So here is at least a suggestion—not to be regarded as proven at all—that the two adrenals may have been making substances in different proportions. Moreover, the pattern is quite different from some of the other cases of Cushing's syndrome, which were all non-tumour cases.

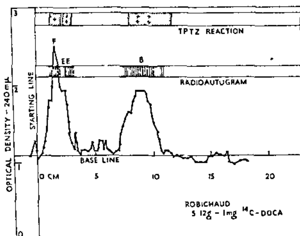


FIG 10

The application of this technique to the case that came in with relatively normal electrolytes (p. 507) is shown in Fig. 10. She had had Cushing's disease for some years, and she was the one who had the marked renal stones and renal disturbance. In her adrenal incubated with ^{14}C labelled DCA, Compound F was by far the highest fraction. The proportions are calculated as follows: F4, E1 and B6. Again let me emphasize that on straight incubation B does not appear in the human gland.

The findings in the case which I mentioned on p. 508, who had the impaired sugar tolerance continuing after the removal

strictly comparable, because it was incubated by the radio-autograph method, after addition of ^{14}C labelled DCA. This gland weighed 9 g., of which 5 46 g. was used. This gland produced the following proportions: B7, E4, F1, X0—that is

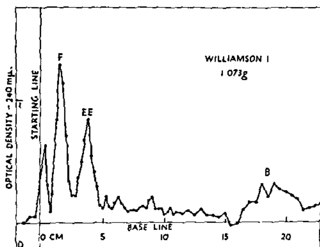


FIG 8.

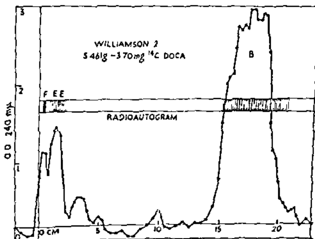


FIG. 9.

bone disease, or who have these serious mental changes, or have not responded to X-ray treatment, should be submitted to surgery. Certainly for any patient who appears to be in reasonably good condition I think a trial on irradiation is justified and ought to be undertaken provided it has been shown that his adrenals are susceptible to stimulation with ACTH and inhibition with cortisone—otherwise one may be

ketosteroids spontaneously, so one has to get a great many determinations to establish a base line, if one only determines the ketosteroids two or three times and then gives cortisone, one can hardly tell whether they are being suppressed or not. I was more impressed with the response Dr. Thorn showed to ACTH than with the cortisone suppression in the Cushing's group. I and Dr. John E. Howard believe that it is almost

with I think Jailer, however, favours the study of ketosteroid suppression. It will be worth while, I believe, to try suppression with some more active compounds, such as the fluorohydrocortisone. But when you have to give 100 mg. of cortisone to suppress the pituitary and are dealing initially with 10–12 mg. 17-ketosteroids, I feel the results are not of much value.

Wilkins: I'm sorry I misinterpreted you. Another question arises as to whether some cases of congenital diffuse adrenal hyperplasia may occasionally go over to an adenomatous condition and then fail to respond to cortisone. I certainly feel strongly that when one does not get suppression of the adrenogenital syndrome with adequate cortisone, then one should explore for tumour.

Browne: Dr Venning, in studying the repression of ketosteroids with cortisone, found one case of a child with adrenocortical adenoma in which the ketosteroids were repressed after about nine days, whereas in most of the hyperplastic cases repression occurs within one to two days

Luft. In the Scandinavian countries we very rarely *see tumours of* the adrenals in Cushing's syndrome. I have seen about 30 cases, and I

of her glands, but no electrolyte disturbance, are shown in Fig. 11. The first gland that was removed was incubated without added labelled DCA. Here the F is quite high and the E considerably less; Dr. Heard calculates the proportions as : F2, E1, X0 and B0.

These results are of course only preliminary and I regret that they are not strictly comparable from gland to gland, but they do indicate the possibility that the two glands, even

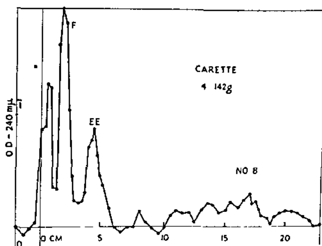


FIG. 11.

when there is no tumour in either and the glands are within the normal limits of size (one 3 g. and the other 8 g.), may differ in their production of steroids. It would be interesting to do further work along these lines and to attempt to correlate urinary excretion, clinical findings, electrolyte and carbohydrate balance, with the production of the gland measured in this manner after removal.

DISCUSSION

THORN: I agree wholeheartedly with Dr. Soffer that a conservative approach is the method of choice in any patient with Cushing's syndrome, for at least two reasons One is that sudden reduction in hormone

Gynaecology has treated a number of these patients with only moderately elevated 17-ketosteroids but otherwise the complete syndrome, with very great improvement and establishment of ovulatory menstrual cycles and fertility. Also Mr. Broster was telling me, and it has been our experience too, that ovarian follicular cysts are not infrequent in cases of adrenogenital syndrome, although not to the same degree as in the Stein-Leventhal. I don't think that Dr. Soffer's data on Cushing's syndrome are evidence against the fact that there might be an adrenogenital factor

Stein-

he
ab
al

one would have to give them more cortisone [illegible]
going through a fairly normal puberty on adrenalectomy or subtotal adrenalectomy. The amount of ketosteroid removed by unilateral adrenalectomy averages about 10 mg. (or 40 per cent) in the pseudohermaphrodites and 50 per cent in the post-pubertal ones.

[illegible]
In that connection, Dr. Houssay has recently been doing partial, fairly complete pancreatectomies in rats, and 70 per cent of the animals develop permanent diabetes. However, if he gives oestrogen, only a

only saw a tumour in one boy of four, fourteen years ago. Generally w don't see adenomas, sometimes hyperplasia, mostly normal adrenal Generally we treat these patients with irradiation, and we are no [illegible] hypophysectomy); we used electrocoagulation of the hypophysis. Fou weeks later the patient had her first menstruation in several years, an two months later she was pregnant. This is now three years ago, an the patient is still normal in every respect—she had been completel bedridden before the operation. We now wonder if what we actually di was just to cut the stalk. We must have left normal pituitary tissue, bu on the other hand the pituitary in this case presumably has no connec tion with the hypothalamus. We are going to perform stalk resection in cases of Cushing's syndrome as well as in malignant exophthalmos

FORSHAM: Professor Harris, do you believe that it will make an difference if they cut the stalk?

HARRIS: It depends how it is cut. There are cases in the literature— Dandy's case is often quoted—where the stalk was cut and the patien reproduced and lactated. But if you take the experimental evidence if the connection is broken and *permanently* interrupted by insertion of a foreign body between the cut ends, it's my belief that reproductiv activity ceases.

FORSHAM: What about ACTH though?

HARRIS: This is anticipating my paper tomorrow, but if you block the stalk, so far as we can see you block the neural, the emotional dis charge of ACTH but perhaps not the discharge for systemic stress

BROWNE: In Scandinavia, is the onset of Cushing's syndrome I mentioned with renal stones common?

LUFT: We have two or three with renal stones out of 30

QUERIDO: With regard to the percentage of tumours, I don't support the Scandinavian figures. In 27 cases we have three adenomas, but I must confess that the first adenoma we have seen was about the 20th, so it is difficult to be statistical about these things.

BAYLISS: May I ask if anyone else has had any trouble with dermatitis in patients that have been partially adrenalectomized? We have had two patients, and I have heard of five or six more, who some long time after the operation have developed what appears to be a mild form of exfoliative dermatitis

FORSHAM: In almost all of our 15 cases who had bilateral adrenalec tomy (1 cancer, 2 adenomas, 12 bilateral hyperplasia) there was peeling of the skin like there is after scarlet fever. It takes about six to eight weeks, but it is never really exfoliative.

ELMADJIAN: One of the only two adrenalectomy patients at Wor cester had a recurrent temperature rise, and during that time there were signs of skin disturbance. Penicillin was given, and some days later the skin eruption disappeared

WILKINS: I'd like to bring up the question of the Stein-Leventhal syndrome again. Dr. Georgeanna Seegar-Jones in our Department of

WILKINS: Has anyone observed any cerebral damage after intensive ... of the pituitary? At Guy's Hospital I was shown a patient

QUERIDO. There are a few other things which I should like to bring up. It is our impression that the posterior wall of the sella turcica is decalcified in more than half the cases, but we don't know whether it bears any relation to general osteoporosis.

Then a practical point about this diagnosis with perirenal insufflation.

FORSHAM: When it comes to perirenal insufflation I would agree with you. But there is a new technique known as retroperitoneal pneumography, whereby you give oxygen behind the rectum. The greatest

work-up.

SOFFER: I should like to make a point about this too. The gas injected is not air, but oxygen. The hazards are very much reduced when oxygen is employed as the insufflative gas. During the past several years we have used presacral or retrorectal insufflation which I think is certainly less hazardous. Our total experience extends over approximately 300 patients who have had either perirenal or retrorectal insufflation and, other than a transitory subcutaneous emphysema in a few instances, which alarmed me, but not the patient, we encountered no difficulties.

*GRAY, C. H. and LUNNON, J. B., (1953), *Mem. Soc. Endocrin.* No. 2, p. 64.

islets in those animals. So the presence of a high cortisone level and an inadequate pancreas may in fact protect the islets, which seems to me very odd. I wondered if that might possibly some day be correlated with the clinical work; it is my impression at least that in most instances where the tumour is removed successfully in Cushing's syndrome diabetes disappears even though the impaired sugar tolerance may have been present for many years.

THORN. We have usually seen the diabetes disappear, though in at

with a very poor pancreas, because whenever you have to put them back on cortisone, small amounts like 10–15 mg., you get glycosuria.

BROWNE: You have also reported one case, Dr. Thorn, in which an individual on high doses of cortisone by implant developed permanent diabetes. This individual had Boech's sarcoid and Addison's disease. That pancreas was perhaps damaged originally. So the reverse is possible, I think. And we have three cases of rheumatoid arthritis who have apparently developed permanent diabetes under cortisone therapy.

THORN: It has always seemed to me that potassium deficiency might be important in the genesis of permanent diabetes. This is reminiscent of Astwood's glucose infusion experiment in which he apparently produced permanent diabetes in some animals. However, Dr. Soffer showed quite a few of these cases which don't have very striking evidence of electrolyte disturbance.

marrow stimulation is one of the striking features of Cushing's syndrome.

SOFFER: We did blood volume studies in a small number of our patients with typical plethora, and found no increase in circulating blood volume. As far as the white cells are concerned, half of our patients had a well-defined leucocytosis but 20 per cent had a con-

volume was normal, and so were the intracellular volume, total body fluid, total exchangeable sodium, total exchangeable potassium, bromide space and renal clearance values.

QUERIDO. I want to support the treatment scheme put forward by Dr. Soffer. We were a little afraid to get dependent on cortisone distribution, and we started treatment with unilateral extirpation and immediate irradiation with 3,000 r tumour dose. We have now treated nine cases on that scheme four we have followed for two to six years who are quite satisfactory, four with one-year follow-up who have responded nicely, and one that didn't show any success. I think that is quite a high score.

must apply, and one may still be up in the air as to what is normal variation and what is disease.

BROWNE: It seems to me that here we are in difficulty in other directions than simply the amount produced (or the amount we imagine

[illegible]

[illegible]

Certainly we are all well conditioned now to the differences, as Dr. Browne indicated, of responsiveness of a wide group of individuals to any dose of hormone. On the other hand, I don't think any of us would do anything seriously in regard to the pituitary or adrenal of one of our laboratory workers, for instance, just because one found an occasional high value of steroid So the laboratory would not make the diagnosis in borderline cases, nor would the clinic alone, but a combination of both.

SOFFER My approach has been a conservative one in the sense that

11-oxygenated steroids and then on fractionation studies with ultra-violet absorption he showed the presence in considerable amounts of a fraction which had a maximum absorption at 236 mμ and which we subsequently found to be $\Delta^{3,5}$-androstadiene-17-one. An adrenal tumour was demonstrated on perirenal insufflation and on operation an

Unfortunately we have only encountered six, each of which was a classical case. In only two of those six was the excretion of cortisone, hydrocortisone and tetrahydrocortisone increased In three the excretion of either free cortisone or free hydrocortisone was increased but the tetrahydrocortisone was not; and the total in those cases of tetrahydro, hydrocortisone and cortisone was within normal limits In the last case the E, F and tetrahydrocortisone were within normal limits I should be glad of some comments regarding this observation.

WILKINS: We had the same experience as far as urinary corticoids were concerned. The blood corticoids were of more value.

FORSHAM: Were the conjugated ones included, Professor Gray, or were these just the free compounds?

GRAY: Both free and conjugated. We almost always find that the E and F are in the free fraction while all the tetrahydrocortisone is in the conjugated fraction.

THORN: Our lowest value so far has been 10 mg., and that can be matched by normal individuals on occasions, although normal people ordinarily do not excrete this much from day to day. But I wouldn't doubt at all that there would be Cushing's patients with normal urinary values after the disease had persisted for some time.

BERGENSTAL: Do you feel, Professor Gray, that this may represent a differential detoxification, since the tetrahydro derivatives are so inactive, metabolically speaking?

GRAY That was my feeling. But I think the picture is a very complicated one. I suppose the free compound represents material coming out by glomerular filtration. But I am at a loss to explain the case in which there was no increase

PRUNTY. I think that today a very important point is raised. One always has the question in mind, what are the minimum clinical criteria for making a diagnosis of Cushing's syndrome? You see quite a lot of people with a little hypertension, a questionable moon face, plethora, a few abdominal striae, a little bit hirsute. As far as I know nobody has ever attempted to answer that problem, and I thought there were several experts here today who might like to give their views.

c and the classical

as far as adrenal

If I had a patient

with hypertension and diabetes with high normal values of urinary 17-hydroxycorticosteroids which persisted, I would think that he fell

disappearance or reduction in hormone excretion following irradiation, I would think that would be useful for the patient and good evidence that the patient was at least borderline Cushing's.

FORSHAM. But isn't that also the group in which you are most likely to apply this ACTH test?

THORN: Yes. But the closer one approaches normal the more tests one

THE RECIPROCAL RELATIONSHIP BETWEEN THE THYROID AND ADRENOCORTICAL RESPONSES TO STRESS

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THE present studies may be conveniently discussed under two headings: firstly a description of the method used to measure thyroid activity in the rabbit and the effect of various stresses on such activity; secondly, the results of studies (still in progress) on the effects of pituitary stalk section on the thyroid and adrenal cortex.

Thyroid Activity and Stress Stimuli in the Rabbit

Measurement of Thyroid Activity

The most direct methods of measuring thyroid activity in the conscious animal are those involving the use of radioactive iodine. There are two main ways in which ^{131}I may be used for this purpose, either to measure the rate of uptake of radio-iodine from the blood into the thyroid gland, or to measure the rate at which radio-iodine, present in the gland in organic combination, is secreted back into the blood as radioactive thyroid hormone. The latter method has been used by Brown-Grant, von Euler, Harris and Reichlin (1954) for following the thyroid activity in rabbits over relatively long periods. This technique has the advantages that anæsthesia is not required and that repeated experiments may be performed on any one animal since only small doses of radioiodine are employed. Forty-eight hours after the injection of a tracer dose of ^{131}I the radio-iodine left in the body is almost all in the thyroid gland in organic combination. From this time onwards twice daily measurements are made of the

adrenocortical carcinoma was found. Following removal of the tumour the œdema entirely disappeared, the $\Delta^{3,5}$-androstadiene-17-one vanished from his urine, and the patient was reasonably well for about three

tension, no disturbance of carbohydrate metabolism, none of the usual clinical manifestations of Cushing's syndrome, and whose only complaint was that of œdema.

COPE: The only case of adrenal carcinoma that I have encountered first hand was entirely casually discovered by the fact that I was

multiple metastases.

DELTOUR: As far as determination of ketosteroids is concerned, and especially the ratio between the alpha and beta compounds, one should

syndrome we found 20 mg. by acid hydrolysis and 40 mg. after the

possible that in remissions the cells of the fasciculata are lipid-laden while an exacerbation of symptoms is associated with the alteration of

hormone, is partly excreted in the urine and partly reaccumulated by the thyroid. Reaccumulation of ^{131}I by the thyroid introduces an error into the use of this method. However detailed studies—involving (a) measurements of the thyroid: renal sharing of ^{131}I, and the rate of excretion of ^{131}I during the course of a release curve, and (b) the distribution of ^{131}I following injection of radio-thyroxine—have shown that only about 10 per cent of the radioactivity lost from the thyroid in any one day is reaccumulated as ^{131}I. The average slope of a release curve in the normal rabbit represents a loss of about 17·6 per cent of the thyroid radioactivity per day. After making the correction for reaccumulation this would represent a true loss of about 19·4 per cent per day. The changes in thyroid activity observed in the present studies are outside the range of this possible error.

The slope of the release curve is proportional (apart from the slight error introduced by reaccumulation of ^{131}I) to the $\frac{\text{amount of hormone secreted per unit time}}{\text{amount of hormone in the gland}}$. Providing therefore that the total amount of hormone in the gland remains constant, the slope of the curve may be taken as related to the amount of hormone secreted per unit time, that is to thyroid activity.

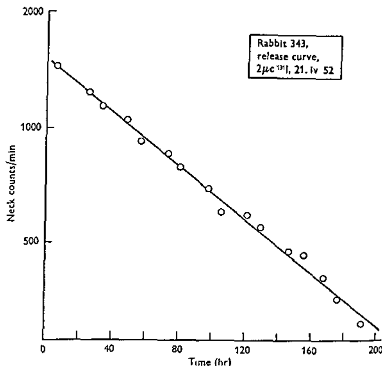
The Effect of Various Procedures, Well Known to Affect Thyroid Activity, on the Slope of the Release Curve.

Hypophysectomy, injection of thyroxine or thyrotrophic hormone, and exposure to cold have been tested on the slope of the release curve.

Hypophysectomy (Fig. 2) produces a marked and permanent decrease in the rate of release of ^{131}I. In one group of normal rabbits the average rate of loss per day was 26 8 per cent, and after hypophysectomy was 3 4 per cent.

Injection of thyroxine (Fig. 3) inhibits thyroid activity by decreasing the secretion of thyrotrophic hormone from the animal's own pituitary gland. *Such injection promptly and* markedly inhibits the rate of loss of ^{131}I from the thyroid.

thyroid radioactivity by placing the animal in a hammock with the position of its head and neck stabilised in respect to a Geiger-Muller tube. After correction for the physical decay of the ^{131}I it is observed that the fall in thyroid radioactivity



Harris and Reichlin, *J. Physiol.*, 1954).

occurs exponentially so that the log. of the neck counts plotted against time gives a straight line (Fig. 1). The slope of this line is related to thyroid activity.

Radioactive hormone secreted by the thyroid is partly excreted in the fæces and partly broken down in the body to liberate ^{131}I into the blood. This ^{131}I, derived from degraded

The Effect of Emotional and Physical Stress on Thyroid Activity.

From time to time reports have appeared in the literature of the effect of stresses, of various types, on thyroid activity. Injection of typhoid vaccine, trauma, fasting, cold, heat (Williams, Jaffe and Kemp, 1949), injection of formalin (Paschkis, Cantarow, Eberhard and Boyle, 1950), injection

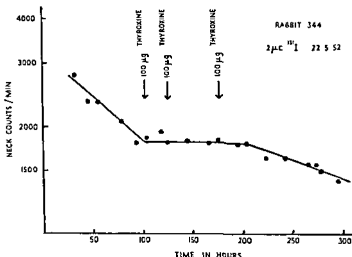


FIG. 3. To show the effect of thyroxine on the release curve of the normal rabbit. Thyroxine probably acts by inhibiting the release of thyrotrophic hormone from the animal's own pituitary gland.

of formalin, cordotomy (Bogoroch and Timiras, 1951), anoxia, starvation (van Middlesworth and Berry, 1951), tourniquet shock (Hamolsky, Gierlach and Jensen, 1951), have all been reported to decrease the uptake of ^{131}I by the thyroid gland of the rat. However measurements of ^{131}I uptake by the thyroid under conditions of stress do not necessarily give a specific index of thyroid activity, since stress (Bogoroch and Timiras, 1951) and injection of cortisone (see Ingbar, 1953) lead to an increased renal clearance of

Injection of thyrotrophic hormone into normal (Fig. 4) or hypophysectomized (Fig. 5) rabbits greatly increases the rate of release of thyroid radio-iodine.

Exposure to cold (Fig. 6) caused a marked increase in the rate of release of ^{131}I from the thyroid in about half the cases in which rabbits were taken from the constant temperature

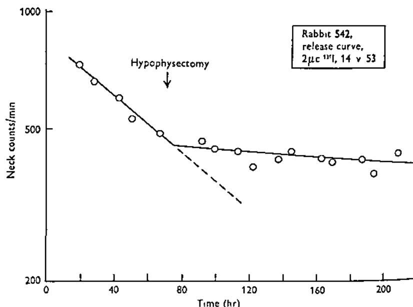


FIG. 2 To show the effect of hypophysectomy on the release curve. Repeated experiments on hypophysectomized rabbits show that the slow rate of release of ^{131}I is maintained indefinitely until the animal is killed. (From Brown-Grant, von Euler, Harris and Reichlin, *J. Physiol.*, 1954).

room (29°C.) and placed in a colder environment. It is of great interest that the stimulus to thyroid activity appeared greater if the animal was placed in an environment at 15–21°C. than if placed at 1°C.

It is clear that the various procedures, which are known to result in definite effects on thyroid activity, have the expected results on the thyroid release curve. It would seem valid then to interpret changes in the release curve, produced by other stimuli, in terms of thyroid activity.

of the release of thyroidal radio-iodine (Figs. 7 and 8). Alternation of two emotional stress stimuli was found to prolong the inhibitory effect on the thyroid, as compared with a single constant emotional stimulus. The interpretation that a decreased slope of the release curve of the stressed rabbit is due to decreased release of thyroid hormone is supported by

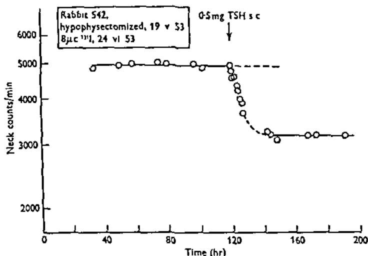


FIG 5. To show the effect of injection of thyrotrophic hormone (TSH) on the release curve of the hypophysectomized rabbit. (From Brown-Grant, von Euler, Harris and Reichlin, *J. Physiol.*, 1954).

the finding that the blood level of organically-bound ^{131}I is simultaneously reduced (Brown-Grant, unpublished).

The mechanism by which stress stimuli result in thyroid inhibition has been investigated. The possibility that this involves a direct nerve supply to the thyroid gland seems unlikely since bilateral removal of the stellate ganglia and a

p

e

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of release of thyroidal ^{131}I, it was thought that the thyroid

iodine, and may thus give an apparent decrease in thyroid uptake due to the fact that less ^{131}I is available to the thyroid.

The effect of emotional and physical stress on the release curve of the rabbit thyroid has been studied by Brown-Grant, Harris and Reichlin (1954*a* and 1954*b*). The emotional stimuli used have consisted of subcutaneous faradism, of

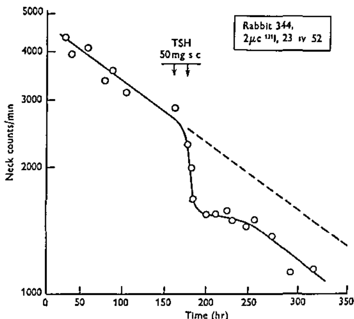


Fig. 4. To show the effect of injection of thyrotrophic hormone (TSH) on the release curve of the normal rabbit. (From Brown-Grant, von Euler, Harris and Reichlin, *J. Physiol.*, 1954)

subjecting the animals to restraint by tying their back legs to the side of their cage or by placing them in small cages which allow them to sit but which restrict their free movement, and of subjecting them to abrupt changes in illumination. The physical stresses have consisted of hæmorrhages, laparotomy under ether anæsthesia, and intraperitoneal injection of turpentine. In the great majority of experiments these stress stimuli resulted in a prompt and marked inhibition

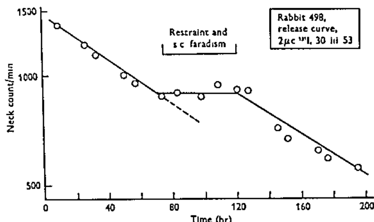
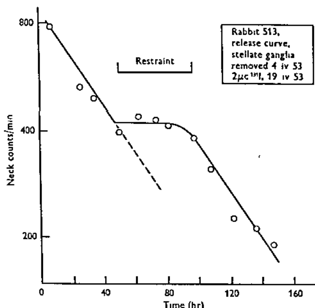


FIG. 7. To show the effect of emotional stress (induced by restraint and subcutaneous faradism) on the release curve of the normal rabbit. (From Brown-Grant, Harris and Reichlin, *J. Physiol.*, 1954*a*).



inhibition might be secondary to adrenal activation by the stress stimuli. However, animals with the adrenal glands denervated, or removed (maintained on constant cortisone regime) showed, in the majority of experiments, thyroid

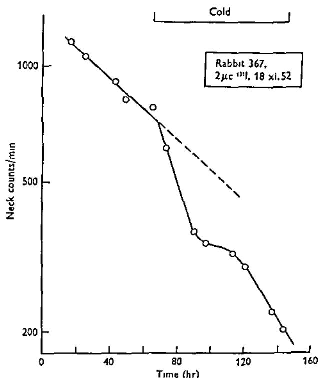


FIG. 6 To show the effect of a cold environment on the release curve of the normal rabbit (From Brown-Grant, von Euler, Harris and Reichlin, *J. Physiol*, 1954).

inhibitory responses to stress stimuli as in normal rabbits. It would seem likely that the inhibition of thyroid activity seen after exposure to stress is due to a decreased secretion of pituitary thyrotrophic hormone. This view is supported by the results of experiments described below.

Injection of cortisone. It is well established that injection of cortisone inhibits, or tends to inhibit, the pituitary secretion of ACTH. Brown-Grant, Harris and Reichlin (1954*b*) found that administration of cortisone to rabbits markedly inhibits the rate of release of ^{131}I from the thyroid, and produced evidence that this effect was due to inhibition of TSH secretion. Injection of cortisone then appears to depress both ACTH and TSH release.

Thyrotrophic secretion would appear to be inhibited under circumstances leading to increased secretion of ACTH. It may also be true that when TSH secretion is increased, ACTH liberation is decreased. As an example of this latter relationship the results seen to follow administration of thiourea or thiouracil may be quoted. Administration of antithyroid compounds results in increased discharge of pituitary thyrotrophic hormone and adrenocortical atrophy (Leblond and Hoff, 1944; Baumann and Marine, 1945; Zarrow and Zarrow, 1951). Since the atrophic adrenal gland of the thiouracil-treated rat retains its sensitivity to ACTH, Zarrow and Zarrow (1951) concluded that thiouracil treatment results in decreased secretion of ACTH. The effect of thiouracil in producing adrenocortical atrophy would seem to be particularly significant since administration of foreign substances so commonly results in increased ACTH discharge.

In view of the reciprocal relationship that appears to hold under circumstances leading to increased discharge of ACTH or TSH, and the fact that the rate of liberation of both hormones can be decreased simultaneously, the possibility exists that these two protein hormones are formed from a common precursor.

The Effect of Pituitary Stalk Section on the Secretion of Thyrotrophic, Adrenocorticotrophic and Gonadotrophic Hormones

The pituitary stalk has been sectioned by a fronto-temporal approach in over a hundred rabbits. Studies have been made on both the adrenal cortex (Fortier, Harris and McDonald;

The Reciprocal Effects of Stress on Thyroid and Adrenal Cortical Activity.

In general, it seems that stress stimuli excite the adrenal cortex and inhibit the thyroid gland, and that this reflects a reciprocal relationship between the secretion of adrenocorticotrophic hormone (ACTH) and thyrotrophic hormone (TSH).

There are two circumstances, however, under which the rates of secretion of ACTH and TSH appear to change in the same direction.

Cold. It is well known that exposure to a cold environment increases the rate of secretion of ACTH, and also increases thyroid activity. It is possible then that cold forms an exception amongst stress stimuli, in so far as it causes an increased rate of secretion of both hormones. However this conclusion does not necessarily follow, since there are few data on record regarding simultaneous measurement of adrenocortical and thyroid activity on exposure to different grades of cold. In the present work Brown-Grant, von Euler, Harris and Reichlin (1954) exposed rabbits to varying degrees of cold and found that ten of twelve rabbits moved from 29°C. to 15-21° C. showed an increased thyroidal ^{131}I output, as compared with two out of seven rabbits exposed to a temperature of 1° C. The only three rabbits in the series to show inhibition of thyroid activity following cold were those placed in a room at 1° C. and subjected to a continuous draught of air. Now exposure to a temperature of about 0° C. is probably a more effective stimulus to ACTH release than exposure to a temperature of 15–21° C. It is thus possible, as suggested by Bogoroch and Timiras (1951), that minor degrees of cold exert a specific effect on TSH release and increase thyroid activity, whereas greater degrees of cold act as a non-specific stress inhibiting the thyroid and activating the adrenal cortex. The effect of cold (5° C.) in inhibiting the thyroid gland has also been observed by Williams, Jaffe and Kemp (1949). Further and more detailed work on this subject is required.

together than in many other species, the breadth of the plate it was possible to insert in this manner was limited (see Fig. 9*b*), and regeneration of the portal vessels has been observed to occur, in some animals, along the sheath of the carotid arteries. Secondly, attempts were made to destroy the median eminence and primary plexus of the portal vessels by means of a d.c. current (usually 3 5 mA. for one min.), applied to the tuber cinereum with a curved platinum electrode. Following this procedure the coagulated stalk was sectioned and a plate inserted. And thirdly, in a further group of rabbits the right internal carotid artery was tied in the neck and one week later this artery was exposed intracranially, ligated and cut, the pituitary stalk cut, one or both oculomotor nerves removed, and a large waxed paper plate inserted under the hypothalamus and secured by pressing between borders of the tentorium cerebelli and clinoid processes. (Fig. 9c).

In presenting the results obtained on these animals they are grouped together and referred to singly as stalk-sectioned rabbits. Since histological examination has not yet been made of the pituitary stalk region in all cases, it is impossible to say in which animals, if any, regeneration of the hypophysial portal vessels has occurred

The Effect of Stalk Section on Thyroid Activity

There are few accounts in the literature in which direct measurements have been made of thyroid activity following pituitary stalk section. In the present work the forty-eight-hour uptake of ^{131}I by the thyroid and the rate of release of radioactive hormone from the gland of stalk-sectioned rabbits have so far been compared with normal and hypophysectomized controls. The values for these three groups of animals are shown in Table I.

The Effect of Stalk Section on the Thyroid Response to Stress

The effect of various procedures on the activity of the thyroid gland has been investigated in twenty pituitary

unpublished) and on the thyroid gland (Brown-Grant, Harris and Reichlin; unpublished) of each animal in a group of about twenty of these stalk-sectioned rabbits. Since these studies are still in progress, and some of the animals reported on are still alive, the results presented cannot be regarded as definitive although they seem sufficiently homogeneous to form at least a basis for discussion.

Operative Procedure.

After exposing and cutting the pituitary stalk several procedures were adopted in an attempt to prevent regenera-

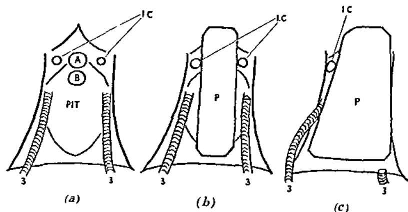


Fig. 9. (*a*) Diagram of the view, from above, of the pituitary gland of the rabbit. (*b*) View of pituitary in sella turcica with a plate inserted between the internal carotid arteries. (*c*) View of the plate covering the pituitary inserted after intracranial ligation of the right internal carotid artery and removal of the right oculomotor nerve

tion of the hypophysial portal vessels across the site of stalk section. Firstly, a waxed paper plate was inserted beneath the optic nerves and slid backwards between the two internal carotid arteries so that it was finally situated beneath the hypothalamus and above the pituitary gland. Since the internal carotid arteries of the rabbit are relatively closer

rabbits in thirteen experiments. On eleven occasions no change in the release of thyroidal ^{131}I was seen. On one occasion a doubtful response and on another thyroid inhibition was observed.

Restraint. In 19 normal rabbits the stress of restraint resulted in thyroid inhibition in 21 of 23 experiments. In 33 experiments on 20 stalk-sectioned rabbits the stress of restraint failed to produce the normal inhibitory response on 27 occasions. On seven (of these 27) occasions however some reduction in the rate of ^{131}I release was observed, which differed from the normal inhibitory response, in that it often started at the end of the thirty-six to forty-eight-hour restraint period and persisted indefinitely, until the end of the experiment. Six rabbits showed a definite decrease in thyroid activity during the stress period. (Four of these six animals also showed a lymphopenia following the stimulus of cold or restraint, see below.)

Effect of Stalk Section on Adrenal Weight

There are many accounts in the older literature on the effect of pituitary stalk section on the maintenance of the adrenal cortex. For example, Brooks (1938) claims that stalk section in the rabbit does not result in adrenal atrophy, and gave figures for the average weight of the left adrenal of 277 mg. in normal rabbits and 276 mg. in pituitary stalk-sectioned rabbits. Much previous work dealing with stalk section is of doubtful significance since precautions were not taken to prevent regeneration of the hypophysial portal vessels. T[illegible] now good evidence that such regeneration occurs in [illegible], that it occurs rapidly and may be related to the [illegible]al anterior pituitary function. From this point [illegible]work dealing with pituitary transplants giv[illegible] regarding the adrenocorticotrophic fun[illegible]itary gland when removed from [illegible]ers who studied the adrenal [illegible] animals bearing pituitary [illegible]l atrophy.

stalk-sectioned rabbits. (Eighteen of the animals were also studied for adrenocorticotrophic responses.) The four different types of stimuli used were laparotomy, injection of thyroxine or stilbœstrol, and restraint (as described above).

Table I

FORTY-EIGHT-HOUR UPTAKE OF ^{131}I AND THE RATE OF RELEASE OF THYROIDAL RADIO-IODINE OF NORMAL AND COMPLETELY HYPOPHYSECTOMIZED RABBITS, AND OF THE STALK-SECTIONED RABBITS DISCUSSED IN THIS PAPER

	Normal rabbits	*Stalk-sectioned rabbits*	*Hypophysectomized rabbits*
48-hr. thyroid uptake of ^{131}I, expressed as a percentage of the dose administered	22·3% ± 0·57* (79 expts., 25 rabbits)	11·3% ± 0·65* (64 expts., 17 rabbits)	8·5% ± 0·95* (20 expts., 10 rabbits)
Slope of release curve—expressed as the percentage loss of thyroidal radioactivity per day.	17·6% ± 0·78* (100 expts., 30 rabbits)	7·9% ± 0·80* (131 expts., 17 rabbits)	3·4% ± 0·55* (32 expts., 10 rabbits)

* ± S.E. of the mean

Laparotomy. Twelve stalk-sectioned rabbits were subjected to laparotomy whilst on a release curve. In ten cases thyroid inhibition was observed, in one case a doubtful response and in one case no response was seen.

Injection of thyroxine. In twenty-three experiments on 19 stalk-sectioned rabbits 100 μg. thyroxine was injected (I.P. or S.C.). Definite inhibition of the thyroidal release of ^{131}I occurred on 20 occasions. In two experiments the response was doubtful and in one, negative. All 19 animals showed a definite response to thyroxine on at least one occasion.

Injection of stilbœstrol. It has been shown (Brown-Grant, unpublished) that subcutaneous injection of 5 mg., or less, of stilbœstrol resulted in thyroid inhibition in forty out of forty-two experiments on normal rabbits. Subcutaneous injection of 5 mg. stilbœstrol has been made into twelve stalk-sectioned

rabbits out of 19 was a slight lymphopenia observed to follow cold or restraint. [Four of these five rabbits also showed (see above) inhibition of the thyroid gland in response to restraint.]

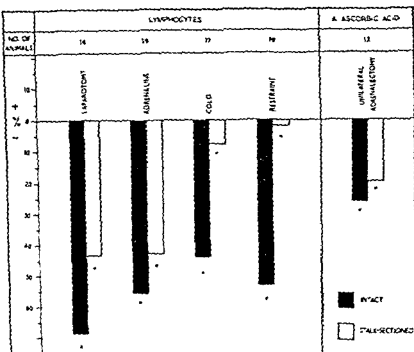


FIG 10. To illustrate the average responses of normal and stalk-sectioned rabbits to the various types of stress stimuli.

The Effect of Stalk Section on the Reproductive Organs

The reproductive organs have been examined in 13 of the animals in which study has been made of the thyroid and adrenocortical responses. In 11 of these animals the ovaries and uteri were atrophic, in one partial maintenance was observed and in the other the organs were in an œstrous state.

The two animals in which some maintenance of the reproductive organs was observed, had been found to give

In the present work the adrenal weight of rabbits in which the pituitary stalk has been sectioned, and precautions taken to prevent portal vessel regeneration, has been studied. The mean adrenal weight (both glands) of normal rabbits was 505 ± 42 (S.E. of the mean of 13 animals), and the mean adrenal weight of stalk-sectioned rabbits was 283 ± 26 (S.E. of the mean of 12 animals). This latter figure is only slightly higher than that found in hypophysectomized rabbits in previous experiments.

From this study there can be little doubt that the hypothalamo-hypophysial connections are intimately related to the normal maintenance of the adrenal gland.

The Effect of Stalk Section on the Adrenocortical Response to Stress

The effect of various types of stress stimuli have been investigated in 19 stalk-sectioned rabbits. The stimuli used have consisted of procedures designed to produce (1) tissue trauma and metabolic disturbances [laparotomy, injection (s.c.) of 500 μg. hyperduric adrenaline (Allen & Hanburys, Ltd.)], or (2) predominantly nervous or emotional excitation (exposure to cold, and immobilization in a hammock). A fall in blood lymphocytes at the third hour after the beginning of the stressful procedure was taken as the criterion of adrenocortical activation. As a further test of the adrenocorticotrophic response to trauma, the ascorbic acid concentration of the right adrenal gland was compared with that of the left gland which had been surgically removed one and a half hours previously. This has been performed, as a terminal experiment, on 12 of the stalk-cut rabbits.

As may be seen from Fig. 10, which gives the average responses for normal and stalk-sectioned rabbits, the operation of cutting the pituitary stalk only slightly reduces the ascorbic acid response to surgical trauma, or the lymphopenic response to laparotomy or injection of adrenaline. However stalk section was found to markedly reduce, or abolish, the lymphopenia that follows cold or restraint. In only five

neurogenic stimuli were no longer effective. At the present time the evidence indicates that stressful procedures may affect the discharge of ACTH and TSH either by an effect mediated through the central nervous system and the hypophysial portal vessels, or by producing a change in the composition of the circulating systemic blood, or by both processes.

The present results of Fortier, Harris and McDonald, that systemic stress still causes ACTH discharge after interruption of the hypothalamo-hypophysial connections is difficult to reconcile with those reported by Hume (1952), McCann (1953) and Porter (1953), in which hypothalamic lesions abolished the adrenocorticotrophic response to surgical trauma or injection of adrenaline. At the moment there is no obvious explanation of this discrepancy.

It is of interest to speculate on the overall function of the pituitary stalk in regard to anterior pituitary function. Dr. C. Fortier has suggested (personal communication) that the stalk forms, essentially, a connecting link between the external environment and the adenohypophysis and its target glands. The adrenocortical and gonadal atrophy and decreased activity of the thyroid gland that follow stalk section might then be explained in terms of loss of the effect of the multitudinous stimuli to which the organism is continuously subjected from its surroundings Since the reproductive organs are as atrophic in pituitary stalk-sectioned animals as in hypophysectomized animals, it would seem that gonadotrophic secretion is more completely dependent on the hypothalamus and pituitary stalk than is the secretion of TSH and ACTH. (The fact that, in the present studies, stilbœstrol no longer inhibited thyroid activity in the stalk-sectioned animal, though thyroxine was still effective, also suggests a closer functional relationship between the hypothalamus and the gonads than between the hypothalamus and thyroid gland.) It is felt, however, that the view that the hypophysial stalk functions as a key link between the external environment and anterior pituitary, and that a

lymphopenic responses to cold or restraint, and thyroid inhibition to restraint.

Discussion

The present results demonstrate that pituitary stalk sections, with steps taken to prevent vascular regeneration, result in—

A marked decrease in activity of the thyroid, adrenal cortex and ovaries.

A marked reduction, or abolition, of the effect on the thyroid and adrenal cortex of stress stimuli that act predominantly through nervous or emotional excitation.

A marked reduction, or abolition, of the thyroid inhibition that follows injection of stilbœstrol. Inhibition of thyroid activity still follows injection of thyroxine.

Only slight diminution of the thyroid and adrenocortical response to the stress of surgical trauma, or of the adrenal response to injection of large doses of adrenaline.

As stated above, the work on which these results are based is incomplete. Serial sections through the pituitary stalk region have been studied in only five of the rabbits. In view of this, and of previous results (Harris, 1950; Donovan and Harris, *unpublished*) in which some regeneration of hypophysial portal vessels has been observed to occur round plates inserted between the cut ends of the pituitary stalk in rats and ferrets, it is possible that completion of the present study may reveal a lack of homogeneity in the animals here grouped together.

Several of the present findings are in good agreement with previous reports. The marked reduction, or abolition, of the lymphopenia produced by emotional stress stimuli, after destruction of the hypophysial portal vessels, was observed in rabbits by de Groot and Harris (1950). In experiments on hypophysectomized rats bearing pituitary transplants in the anterior chamber of the eye, Fortier (1951) observed that systemic stress stimuli still resulted in an eosinopenia, whereas

THE POSSIBLE FUNCTIONAL SIGNIFICANCE OF THE PITUITARY PORTAL VESSELS

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Introduction

THE hypothesis that the pituitary portal vessels play a critical part in a "mechanism" whereby the hypothalamus directly controls the pars distalis of the pituitary is essentially based on the three following propositions:

1. Such a control exists.
2. No nerve fibres or any continuous structural elements other than blood vessels link the hypothalamus through the pituitary stalk with the pars distalis.
3. The anatomical basis of the control must, therefore, be the hypophysial portal vessels which connect the median eminence above with the pars distalis below; and the means whereby the control is exercised must be some chemical secretion(s) (as yet neither isolated nor identified) of hypothalamic neurons (unspecified) which diffuses into the primary capillary network of the pituitary portal vessels.

The acceptance of this argument would seem to imply that when the pituitary gland is effectively separated from the hypothalamus (e.g. by grafting or by stalk section), the *functions which are alleged to be controlled by the hypothalamus* would become deranged. Were this not so, the inference would be that the two structures are not connected functionally, or at any rate not in the way envisaged.

Some other observations which add up to the general view that certain pharmacological agents, e.g. adrenaline, stimulate the anterior pituitary, while others, e.g. dibenamine, "block" its responses, are often used to support, or even thought to establish, the hypothesis that the hypothalamus controls the

type of "disuse atrophy" of the target glands occurs after stalk section affords a general summary of available knowledge.

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[Discussion of this paper was postponed until after the paper by Prof. Zuckerman—*Ed*].

Others find that the gland's reactions are affected by lesions in the median eminence, which is not part of the hypothalamus proper, but essentially a component of the neurohypophysis. Thus McCann (1953) states that the median eminence is the only site where lesions affected the ACTH response of the pars distalis; and further, that the latter is unaffected by damage to any of the hypothalamic nuclei that are known to send fibres into or through the eminence. If this is indeed the case, the subsequent hypo-function or non-function of the anterior pituitary is far less likely to be due to the interruption of some form of transmission from the hypothalamus, than to vascular damage in the pituitary portal system of vessels leading to infarction and lasting pathological changes in the anterior pituitary (see below).

The second proposition

Since, apart from blood vessels, there are no possible continuous elements other than nerve fibres which could connect the anterior pituitary below with the hypothalamus above, the second proposition on which the hypothesis is based concerns only the question of the presence in the pars distalis of the axons of hypothalamic neurons. The prevailing view that none exist cannot be regarded as final. The conflicting literature on the subject is reviewed by Harris (1948), who, while not assessing the technical merits of the papers to which he refers, sums up his own impressions in the statement that "the hypothalamic innervation of the adenohypophysis appears scanty if it constitutes a true secreto-motor nerve supply to the organ". At the Ciba Foundation Colloquium on Anterior Pituitary Secretion, 1952, he put the somewhat more

pituitary stalk, and he specifically says that the ventro-medial nuclei are generally undamaged. *Alloiteau and Courvoisier (1953a and b) appear to have*

the median eminence, and involving the n supraopticus and anterior hypothalamus.

pituitary in the way suggested. In fact, the interpretation of these observations is still questionable. Nor would the acceptance of the hypothesis enable us to predict them. In so far as they imply specific actions of pharmacological agents, and to the extent that they can be reconciled with each other, they stand equally well on their own, or could be related to almost any speculation about the way the hypothalamus controls the anterior pituitary. It is only because of a prevailing fashion that this particular set of observations is to-day usually associated with the hypothesis in which the pituitary portal vessels play a part.

The first proposition

It is sufficient to say of the proposition on which the hypothesis is based that there are many clinical and some experimental observations which indicate that lesions in, or stimulation of, various parts of the hypothalamus may be associated with disturbances or activation of physiological processes in which the hormones of the anterior pituitary are concerned. On the other hand, these observations incorporate *many inconsistencies, and as yet there is no clear evidence* that specific lesions in, or stimulation of, specified hypothalamic nuclei have constant physiological effects on anterior pituitary function. It is an understatement to say of these differences, as McCann (1953) has done, that we are merely dealing with "incomplete agreement as to the specific anatomic localization". For if the anatomy of the hypothalamus and median eminence has any meaning at all, some of the available reports on the functional relation of the hypothalamus to the pars distalis are totally irreconcilable. For example, different workers describe the same changes in anterior pituitary function as resulting from lesions in, or stimulation of, quite different nuclei in the hypothalamus.*

capillary network, which is in the median eminence, is fed by branches of the superior hypophysial arteries, and it drains into large portal vessels which open into the sinusoids of the pars distalis. The only two points at issue here are (1) the direction of their flow, and (2) the extent to which they contribute to the blood supply of the pars distalis.

The original belief (Popa and Fielding, 1930) that the flow was from the gland to the median eminence proved to be incorrect in the light of the more extensive anatomical studies of Wislocki and King (1936), and on the observations on the direction of flow made by Green and Harris (1949), and by Barrnett and Greep (1951*a*). Recently, however, it seems to have been resurrected, even if in a somewhat different connection, by Spatz, Diepen, and Gaupp (1948), and by Nowakowski (1952). But the balance of evidence, in spite of the fact that in the most direct experiments the flow was observed under conditions which may have greatly transformed the normal hydrodynamic pressure relations, is still clearly in favour of the view stated by Wislocki and King.

The second question seems somewhat more open. In a careful series of studies beginning in 1936, Wislocki and King confirmed the well-known observation that the pituitary gland is supplied from below by two inferior hypophysial branches of the internal carotid arteries, which immediately penetrate the neural lobe; and from above by a series of superior hypophysial branches which spring from the circle of Willis. Some of the superior arteries pass directly into the pars distalis, while others break up into the primary capillary network of the pituitary portal system within the pars tuberalis and median eminence. This network drains into a series of portal vessels which pass along the stalk into the sinusoids of the pars distalis. According to this conception the latter are, therefore, fed both by arterioles which reach them directly, and by the portal channels. Wislocki and King also point out that the vessels which supply the pituitary anastomose freely with one another, forming a vascular continuum, and that there is only a slight capillary connection between

rigid view, that "there is no sound evidence for believing that the pars distalis of the pituitary receives secreto-motor nerve fibres". At the same meeting, however, Vazquez-Lopez and Williams (1952) gave an account of a study of the pituitary of the rat, stained by means of a modified Cajal technique, which indicated that many nerve fibres from the hypothalamus run into the pars tuberalis and on to the pars distalis. Commenting on this finding, Harris suggested that Vazquez-Lopez and Williams might have confused reticular connective tissue with nerve fibres, and at the same time rightly pointed out that the critical experiment which would show whether hypothalamic nerve fibres are present in the pars distalis would be to determine whether the density of the presumed fibres is reduced after the pituitary stalk is cut. This experiment had not been performed by Vazquez-Lopez, but as Harris had himself noted in his 1948 review, it had already been carried out on rats and rabbits by Brooks and Gersh (1941). These workers reported that the density of nerve fibres in all parts of the pituitary is much reduced in the rabbit after stalk section, and apparently to some extent also after superior cervical ganglionectomy. The observations which they made on the rat were in general indecisive. More recently, Metuzāls (1954) has studied the pituitary of the horse, using Bielschowsky-Gros preparations, and has found nerve fibres, probably originating in the hypothalamus, which traverse the infundibulum and pars tuberalis on their way to the pars distalis.

It is obvious that categorical denials of the existence of hypothalamic axons in the pars distalis—whatever their possible function—are premature, and that more work on the subject is called for, particularly studies of the kind already attempted by Brooks and Gersh.

The third proposition

If a doubt still lingers about the existence of hypothalamic nerve fibres in the pars distalis, none need be entertained about the reality of the pituitary portal vessels. Their primary

These workers hold that the so-called loral artery* has a particular significance as an anastomotic channel between the superior and inferior hypophysial arterial systems. On the basis of arterial injections with neoprene latex and Berlin Blue, they also claim that the sinusoids of the pars distalis are filled only *via* the portal vessels, and that in spite of a relatively long course through the pars distalis, the "artery of the trabecula" (loral artery) gives off no branches to its glandular elements. Instead, it helps to feed a complex capillary network in the lower part of the pituitary stalk ("lower infundibular stem" in their terminology), which in turn drains by "short hypophysial portal vessels" into the sinusoids of the posterior part of the pars distalis. The

pituitary stalk which lies above the diaphragma sellæ. Xuereb *et al.* emphasize the richness of the inter-arterial anastomosis around and within the pituitary gland, and suggest that blood can be distributed to all the components of the pituitary body, other than the glandular cells of the pars distalis, through either the superior or inferior hypophysial arteries. *Presumably, therefore, the secretory tissue of the* anterior lobe is supplied from both arterial systems too—since both feed the capillary bed from which the portal veins arise.

The detailed descriptions that are embodied in these two new reports illustrate the great individual variability which characterizes the exact disposition of the vessels of the pituitary. But it is difficult to tell whether the anatomical facts that are reported justify all the generalizations to which they have given rise. This comment applies particularly to the possibility that viscous *injection masses (e g. neoprene latex)* do not necessarily provide an adequate means of deciding

the primary capillary network of the pituitary portal system and the hypothalamic vasculature. Another important point which they note is that no veins accompany the superior hypophysial arteries.

This picture of the vascular anatomy of the pituitary has been modified in recent years, and the view is gradually taking hold that in mammals, as in birds and lower vertebrates, no branches of the superior hypophysial vessels pass directly to the sinusoids of the pars distalis. All the blood in these arteries is believed to be shunted through the primary capillary network in the median eminence before reaching the pars distalis (Green, 1951).

New details have recently been added by McConnell (1953), who studied the human pituitary. This worker draws attention to certain "parallel vessels" in the lower part of the pituitary stalk. Some of these are given off by a branch of the inferior hypophysial arteries called the "interlobar artery". Others arise from a series of "long stalk arteries" which are branches of the superior hypophysial arteries, and which course down just beneath the surface of the stalk. The parallel vessels are said to communicate not only with the capillary network of the neural process but also with sinusoids in the pars distalis. The superior hypophysial arteries also give off a pair of vessels, the "loral" arteries, which pass down the arachnoid space to the front of the stalk, and which enter the pars distalis independently. There they unite with the long stalk arteries just in front of the terminal part of the stalk, and they anastomose with the interlobar branch of the inferior hypophysial arteries. The superior hypophysial arteries also give off a series of "short stalk arteries". These feed a complex network of capillary vessels in the median eminence and stalk. The network is drained by 20 or so large portal vessels (called "sinuses" by McConnell), and these are the main afferent supply of the sinusoids of the pars distalis.

The essentials of this newer picture have been largely confirmed in a still more recent study of the human pituitary carried out by Xuereb, Pritchard and Daniel (1954*a* and *b*).

change in the pituitary itself—for we have no means of observing or measuring changes of this kind in the living animal. Indeed, the knowledge is not even available by which to infer, post-mortem, what changes in pituitary function would have been correlated with what cyto-architectural changes in the gland. The derangement would be revealed in the disorganization of such processes as the release of LH in the female rabbit after mating; in the failure of œstrus to supervene in the anœstrous ferret after exposure to light; or in a lack of ACTH discharge after an animal had been exposed to what is called emotional stress. These indices are precisely the same as those which characterize pituitary hypo-function or non-function—whatever their cause. It is nevertheless claimed that there is a specific correlation between derangements of this kind and division of the pituitary stalk, provided that the operation is not associated with regeneration of the pituitary portal vessels (Harris, 1950, 1952; Harris and Jacobsohn, 1952; Benoit and Assenmacher, 1953*a* and *b*). Harris is also of the opinion that published reports which indicate that division of the pituitary stalk has not been associated with derangement of anterior pituitary function imply that the portal vessels had regenerated in the experiments concerned, and had re-established a vascular link between the median eminence and the pars distalis.

If the only difference between animals with deranged and those with normal pituitary function was the existence or non-existence of such a vascular link, the correlation underlined by Harris would be the strongest possible evidence in favour of the hypothesis in which the pituitary portal vessels have been given the central rôle. But if any other factors were involved, even to a variable extent, the correlation, or apparent correlation, would not necessarily carry the implication which Harris is concerned to emphasize. Now, since the pituitary portal vessels play so important a part in the blood supply of the pars distalis, it is evident that their effective division would not only interrupt a possible channel for the

whether or not, in its course through the pars distalis, a vessel such as the artery to the trabecula (= loral artery) sends small branches into the sinusoids by which it is presumably surrounded. Furthermore, both researches were made on post-mortem material, and both draw attention to the fact that only partial fillings of the arterial system were usually obtained in individual cases—presumably at least partly because of post-mortem clotting. The picture of the vascular anatomy of the pituitary to which they lead was thus actually built up from independent observations of a number of specimens, practically all of which differed in the details of their external and internal vascular structure. Whatever the final story is, there can, however, be no doubt that the pituitary portal vessels play a major part in the blood supply of the gland.

The second part of the third proposition is speculation only, and need not detain us No-one has isolated or identified the presumed chemical substance(s) which are supposed to activate the anterior pituitary, and which certain unspecified hypothalamic neurons are supposed to produce. Nor has there been any discussion of the question whether the same degree of specificity applies to these assumed hypothalamic secretions as supposedly does to those of the anterior pituitary. Apart from extracts with the properties of posterior-lobe hormones, the only pharmacological actions of hypothalamic tissue that have so far been reported are completely non-specific (cf. Zetler, 1953; Hild and Zetler, 1953; Vogt, 1953, Harris, Jacobsohn and Kahlson, 1952).

The test of the hypothesis

As already noted, acceptance of the argument that the pituitary portal vessels are an elective pathway for some presumed chemotransmitters by which the hypothalamus controls certain functions of the anterior lobe, would imply that when the gland is effectively separated from the hypothalamus these functions would be deranged. The overt manifestations of such derangement would not be any direct

change in the pituitary itself—for we have no means of observing or measuring changes of this kind in the living animal. Indeed, the knowledge is not even available by which to infer, post-mortem, what changes in pituitary function would have been correlated with what cyto-architectural changes in the gland. The derangement would be revealed in the disorganization of such processes as the release of LH in the female rabbit after mating; in the failure of œstrus to supervene in the anœstrous ferret after exposure to light; or in a lack of ACTH discharge after an animal had been exposed to what is called emotional stress. These indices are precisely the same as those which characterize pituitary hypo-function or non-function—whatever their cause. It is nevertheless claimed that there is a specific correlation between derangements of this kind and division of the pituitary stalk, *provided that the operation is not* associated with regeneration of the pituitary portal vessels (Harris, 1950, 1952; Harris and Jacobsohn, 1952; Benoit and Assenmacher, 1953*a* and *b*). Harris is also of the opinion that published reports which indicate that division of the pituitary *stalk has not been associated with derangement of anterior* pituitary function imply that the portal vessels had regenerated in the experiments concerned, and had re-established a vascular link between the median eminence and the pars distalis.

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transport of some presumed chemotransmitters, but would also be associated with a variable, and possibly considerable, amount of vascular damage to the pars distalis itself, and that this of itself might lead to hypofunction. To test the hypothesis of the portal vessels, it is, therefore, necessary to transform it into the positive question: is a vascular connection between the median eminence in the floor of the hypothalamus either (a) sufficient, all other factors being the same, or (b) necessary for those responses of the anterior pituitary in which the hypothalamus appears to be concerned? Both questions can be answered only by recourse to experiment in which the anterior pituitary has been successfully transplanted, or in which the anterior pituitary's vascular connections with the median eminence have been effectively interrupted. And since it is the usual experience that pituitary grafts do not "take" successfully, and that hypofunction of the anterior pituitary occurs after transection of the stalk, it is essential to focus attention on those instances where function has appeared not deficient but normal after these operations. For to establish or reject the hypothesis on the basis of observations of "no-response" would be to try to prove a negative. Whereas the establishment of a single case which showed that a vascular connection between the base of the brain was neither sufficient, other things being equal, nor necessary, for positive responses of the kind we are discussing would necessitate the rejection of the hypothesis—at any rate so far as the pituitary portal vessels are concerned. A scientific hypothesis falls to the ground the moment it is proved contrary to any one of the facts for which it is designed to account.

I propose, therefore, to turn to certain experiments, some reported in the literature, and others carried out in my own laboratory, which help in a critical test of this kind.

Pituitary grafts

The literature dealing with auto- and homografts of the pituitary gland is considerable, and more than anything else

bears witness to the fact, unfortunately also confirmed by the experience of my own laboratory, that such grafts are usually unsuccessful—as indicated by involution of the gonads, adrenal cortex and thyroid glands, and reduced growth in the hypophysectomized hosts. Such failures could be due to a multiplicity of factors, including tissue immunity reactions and simple failure of vascularization. Another common and unfortunate source of experimental failure in cases where a graft may have taken is that fragments of anterior pituitary may have been left in the sella turcica of the presumed hypophysectomized host animal. Of the many accounts of pituitary grafts that have been published, the following appear to report positive results that are relevant to the hypothesis under examination.

(a) May (1935) hypophysectomized two male rats by the usual technique, and in the weeks following the operation noted that growth had ceased, and that the testes began to regress. Homografts of the pituitary glands of day-old rats were made into the anterior chamber of the eye two months later. Within a few days the fur of the two rats had become smooth, and their testes redescended into the scrotal sacs. The animals also began to grow and become more active. They did not, however, reach the weight of the controls. One of the two animals died after one hundred and forty-three days and no post-mortem was possible. The other was then killed, one hundred and thirty-five days after grafting, and histological study showed that hypophysectomy had been complete, and that the graft in the anterior chamber was well vascularized and normal in size. The testes were normal in structure. These two animals were apparently two exceptional "positive" cases among an unspecified number of failures.

In a further study, May (1937) reports successful homografts of the pituitary glands of day-old rats into the anterior chamber of two female rats that had been hypophysectomized four weeks before. Within a few days after the operation, the animals resumed normal growth, their coats became smooth, and they became more active. Eight to eleven weeks

later normal œstrous cycles recommenced. Because of this delay May concluded that the anterior pituitary gland of the new-born rat has to become mature before it manifests any gonadotrophic action.

Seven weeks after the appearance of the first œstrus a mature male rat was introduced to one of the females with a graft, and also to a control rat. Both became pregnant, and bore young, but the animal bearing the graft failed to nurse her litter, and nine days later was again in œstrus. One hundred and seventy-five days after the graft was made, the eye bearing the transplant was removed, together with one ovary and associated tube. Histological study showed that the grafted pituitary tissue was normal from the cytological and vascular points of view, but disorganized structurally. The ovary and tube were normal. In the two weeks which followed, this animal underwent two phases of œstrus before subsiding into a condition of continuous diœstrus, and eight and a half weeks later it was killed. Histological examination confirmed the total absence of pituitary tissue in the sella turcica, and showed that the remaining ovary had involuted.

Another hypophysectomized rat treated in the same way also resumed œstrous cycles after the grafting of a pituitary gland into the anterior chamber of the eye. This animal was sacrificed five months after the transplantation, and the ovary and Fallopian tube were found to be normal but smaller than those of its control. No pituitary tissue was found in the sella turcica.

May's descriptions indicate that he was thoroughly aware of the need to establish the completeness of the hypophysectomy in his grafted animals. His observations thus show that periodic œstrous cycles can occur in animals in which there is no direct connection of any kind between the pars distalis and the hypothalamus. The fact that one of his two animals manifested two cycles (? phases of œstrus) after the removal of the pituitary gland is not necessarily inconsistent with the observation that the animals were completely hypophysectomized. A vaginal smear typical of œstrus may

occur at least once after hypophysectomy in rats, and in our own experience is to be expected if the operation is performed in late diœstrus. This is also the case when bilateral ovariectomy is carried out during that phase of the cycle.

(b) Martins (1936) made homografts of the pituitary glands on to the kidneys of four female rats weighing between 50 and 85 g., sixteen to one hundred days after their pituitaries had been removed. One of the four animals underwent four cycles in forty-seven days. Martins stated that he fully established that there was no pituitary tissue in the sella turcica, but does not say whether this observation was made on the basis of histological study of serial sections of the sella turcica. He does, however, state that histological examination showed that the thyroid and suprarenal glands in the animals concerned were involuted.

(c) Successful homotransplants of anterior pituitary tissue are also reported by Schweizer, Charipper and Haterius (1937), who used adult female guinea-pigs in their studies. The animals were hypophysectomized by the parapharyngeal approach, and homotransplants of anterior pituitary tissue were made into the anterior chamber of each eye. The animals were sacrificed three to thirteen weeks after the operation, and reproductive organs, thyroids and adrenals studied histologically. The sella turcica was not serially sectioned, but the authors state that "in every instance the sella was scraped and any fragments found were carefully removed and studied in serial section; only those cases in which no trace of anterior lobe tissue existed in the sella are included in this report". In the cases reported, "the ovaries became progressively more follicular until after approximately 2 months a condition of constant estrous supervened" with the uterus greatly hypertrophied. The ovaries did not contain any well-defined luteal bodies, and there was no sign of ovulation Since no cyclical reproductive changes were observed, it was concluded that the pituitary implants were secreting FSH and not LH. The grafts also maintained the thyroids and adrenals in an apparently normal condition.

In a later study on the male guinea-pig, Schweizer, Charipper and Kleinberg (1940) reported that homografts of anterior pituitary tissue into the anterior chamber of the eye not only maintained full spermatogenesis, but also healthy interstitial elements, since the accessory reproductive organs were in a normal condition.

(d) Cutuly (1941) has reported successful autografts of anterior pituitary grafts into the anterior chamber of the eye of six male rats, and into the sella turcica of four others. The completeness of hypophysectomy was ascertained "by microscopic examination of serial sections of the capsule of the pituitary", and Cutuly restricted his report to animals "known to be completely hypophysectomized". The animals were kept under observation for up to two hundred and fifty-eight days. Four of those with successful ocular grafts continued to grow, and spermatogenesis and androgen production occurred in all except one of the animals. One rat "twice had fertile matings, and sired normal litters". On the other hand, none of the rats bearing intraocular transplants showed evidence of adrenal or thyroid stimulation. "Moderate but distinct stimulation" of the adrenal gland did, however, occur in four rats bearing the successful sellar grafts.

(e) Contrary to Cutuly's observation about the adrenal glands, McDermott, Fry, Brobeck and Long (1950) have observed that intraocular homografts of pituitary tissue not only have the ability to release ACTH both spontaneously and in response to stimulation by systemic or local epinephrine, "but also release steadily enough ACTH to support, at least partially, the weight of the adrenal glands". The completeness of hypophysectomy in the experiments on which this conclusion is based was indicated by the fact that epinephrine did not produce an eosinopenic response after enucleation of the eyes bearing the grafts.

(f) Similar results have been reported by Cheng, Sayers, Goodman and Swinyard (1949) in a study of hypophysectomized male rats with viable anterior pituitary grafts in the anterior chamber of the eye, spleen, or sella turcica. These

glands did not prove capable of supporting growth, but animals were maintained at their original weight. In only one animal was the gain in weight sufficient to suggest that it was due to growth hormone produced by the pituitary transplant. The weight of the testes in the animals bearing viable grafts indicated that the latter were not producing gonadotrophic hormone. With the exception of one animal with a sellar graft, all the operated animals also had atrophic adrenals, and Cheng *et al.* note that the decrease in adrenal weight was in most instances as great as that which occurs in hypophysectomized animals without pituitary grafts. On the other hand, the animals responded to the injection of histamine by a significant decrease in adrenal ascorbic acid.

The completeness of hypophysectomy was proved by serial sections of the sella turcica and surrounding tissue in only three of the animals. Nevertheless, Cheng *et al.* are confident that the reduction in adrenal ascorbic acid in the other ten animals was not due to the release of ACTH from residual pituitary tissue in the sella turcica. Since a sufficient period had elapsed between transplantation and testing, they are also confident that the results they observed were not due to absorption of the implants. They therefore conclude that the pituitary grafts which they studied were able to release ACTH in response to the stimulus of the injected histamine, and further, that "neural connections or a portal venous system between the hypothalamus and the pituitary are not necessary for the prompt discharge of ACTH in response to stress". Cheng and his co-workers also hold that the fact that the adrenal glands in their experimental animals were small was not due to inadequacy of ACTH function of the graft, but to partial atrophy reflecting a decrease in the requirement for cortical steroids in animals whose metabolism was depressed due to hypothyroidism.

(g) Results similar to those of Cheng *et al.* have also been reported by Fortier and Selye (1949), but these authors relied for their check of the completeness of hypophysectomy

on "careful examination of the sellar region with a 5× magnifying glass".

(h) Cheng *et al.*'s differentiation of the *maintenance of the* weight and function of the adrenal cortex of animals bearing pituitary grafts appears to apply, according to a study reported by Greer, Scow and Grobstein (1953) to the thyroid gland as well. These workers made ocular transplants of pituitary tissue in an inbred strain of mice, and then studied the total accumulation of ^{131}I. The animals were hypophysectomized one week after grafting, and the completeness of hypophysectomy was judged by comparing the responses of the animals with those of hypophysectomized controls. The tests were made three weeks after removal of the pituitary, and post-mortem study showed that while the thyroid, adrenal and gonads were no less involuted, so far as weight was *concerned, in the grafted animals* than in their hypophysectomized controls, thyroidal uptake and concentration of radioactive iodine were the same as in normal animals.

(i) In another recent study, Everett (1954*a* and *b*) reports on six rats which were proved by histological examination to be completely hypophysectomized, and which had had their own pituitaries grafted under the renal capsule. Four days later a thread was passed through the left uterine horn. Since a strong deciduomatous reaction developed in each of these animals, Everett considers that the grafted pituitary was able to secrete and release luteotrophin. Conclusive evidence of this view was obtained in other experiments in which the test of progesterone secretion was vaginal mucification in the presence of œstradiol benzoate. *These also indicated that the release of* luteotrophin can occur up to ninety days after transplantation. In general these observations confirm corresponding findings reported by Desclin (1950), and which show that œstrogen is not only able to affect the histological structure of a transplanted pituitary (in animals which study of serial sections of the sella turcica showed to be completely hypophysectomized), but also to cause it to secrete luteotrophin.

(j) The last study to which I shall refer here is also a recent

one, and deals with growth hormone. Martinovitch (1954) cultivated *in vitro* the pars distalis of rats ten to seventeen days old for periods of one to two months. This cultivated tissue was implanted into rats which had been hypophysectomized at the age of sixty to ninety days. The grafts were made from two to eleven months after the operation, and it is stated that during this period growth had ceased. The animals immediately started growing again, but at a slower rate than normal. The grafts were made into the anterior chamber of the eye, and one is reported to have persisted for thirteen months.

This review of reports of pituitary transplantation has focused essentially on those instances which indicate that grafts remote from the hypothalamus can function normally, even if at a lesser intensity than normal. As already noted, a far larger number of reports could have been cited to bear witness to the fact that the likelihood of homo- or autografts of the anterior pituitary "taking" is not very great. There are also indications that grafts "take" better in some than in other regions, e.g. the sella turcica (Greep, 1936; Cutuly, 1941). Whatever the reason for the technical difficulties which hinder successful transplantation of the pituitary, it is, however, clear that authentic cases do exist of hypophysectomized animals bearing ocular pituitary grafts which manifested what can only be described as normal pituitary function, including cyclical gonadotrophic function.

Pituitary-stalk section

A similar conclusion about normal function can be drawn from reports of experiments in which the pituitary stalk has been divided. But here the study is complicated by the fact that the conditions of our test are not satisfied unless there is no re-establishment of a vascular link between the pars distalis and the median eminence.* I shall, therefore, refer

*It was Harris (1949, 1950) who pointed out that the hypophysial portal vessels may regenerate very easily unless a barrier is placed between the two parts of the divided stalk.

here only to experiments, including some done in my own laboratory, in which this necessary condition has been deliberately observed, or in which steps were taken to find out whether a direct vascular link had, in fact, re-established itself between the median eminence and the pars distalis after the division of the stalk. Harris's own stalk-section experiments (1950), and the corresponding ones made by Westman and Jacobson (1938*a* to *f*) and by Benoit and Assenmacher (1953*a* and *b*) will be considered later.

(a) The first of these studies are those of Greep and Barrnett (1951) and Barrnett and Greep (1951*b* and *c*) on the gonadotrophic, thyrotrophic and adrenotrophic activity of the pars distalis of rats after stalk section. The operation was successfully performed on 50 adult male and 43 adult female rats. In the case of the males it was observed that the testes were retracted into the abdomen within a few days after operation, and that in all but a minority they had involuted to about one-quarter normal size in the space of a month. In the exceptional few, spermatogenesis was still in progress and the interstitial tissue appeared normal. The average testis weight in a group of stalk-sectioned rats killed two months after the operation was, however, greater than that of stalk-sectioned rats killed at thirty-five days, excluding four in which relatively little testicular involution occurred. Assay of the pituitaries of the stalk-sectioned rats showed that they possessed very little gonadotrophic potency. But the gonadotrophic potency of the pituitaries of rats that were both stalk-sectioned and castrated proved to be greater than that of animals which had been subjected only to stalk-section.

Division of the stalk in the female rat led either to suppression or disorganization of the cyclical vaginal changes. After a latent interval of twenty days, a few animals restarted irregular cycles. Post-mortem examination showed that the ovaries had atrophied, but seldom as extensively as after hypophysectomy. The evidence thus suggested hypoactivity rather than total loss of ovarian function.

In both sexes stalk-section depressed growth and led to

involution of the thyroid and adrenal glands—but not as much as after hypophysectomy. Exposure to cold, however, evoked a normal response in both the thyroid and the adrenal cortex. It is worth noting that whereas the average weight of the pituitaries of the stalk-sectioned animals was *less than half* that of the normal controls, the thyroid glands were nearer two-thirds. The average width of the adrenal cortex in the operated animals was also about two-thirds that of the controls; that of the zona glomerulosa was, in fact, greater.

A large number of specimens were carefully examined by serial section, after the vascular system had been injected with Indian ink, *in order to see whether regeneration of the portal* vessels had occurred. No instance of this having happened was observed: "the vessels of the stalk remained interrupted and scar tissue filled the gap overlying the incision in the basisphenoid bone between these vessels and the anterior lobe". This difference from Harris's results Greep and Barrnett suggest may have been due to their different operative approaches.

In general these authors conclude that stalk section leads to panhypopituitarism of varying intensity. This they relate to histological changes in the pituitary consisting of *involution, central fibrosis, and loss of basophils particularly,* but also of acidophils. Small chromophobes become the predominant cell type after the operation. Degenerative changes in the pars distalis are most conspicuous in the few days after the stalk is cut. The partial regeneration which then occurs is associated with a partial return of gonadal function—both in males and females—and the degree of function which the "infarcted pituitaries display may be dependent on the rate and degree of revascularization", which apparently occurs from the surrounding capsule. In short, the responses of the pituitary after stalk section are not altered in kind, but *only in intensity, depending on the degree* of vascular damage suffered by the pars distalis, and not on the "complete interruption of the nervous and vascular connections between the hypothalamus and the anterior lobe.

here only to experiments, including some done in my own laboratory, in which this necessary condition has been deliberately observed, or in which steps were taken to find out whether a direct vascular link had, in fact, re-established itself between the median eminence and the pars distalis after the division of the stalk. Harris's own stalk-section experiments (1950), and the corresponding ones made by Westman and Jacobson (*1938a* to *f*) and by Benoit and Assenmacher (*1953a* and *b*) will be considered later.

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between the vessels in the two structures. In the remaining three there may have been an anastomosis through intervening scar tissue, but there was no anatomical reason for supposing that blood from the remains of the portal vessels would not have passed through the scar tissue into neighbouring venous channels and so directly into the systemic circulation. A platelet of waxed paper had been inserted between the divided ends of the stalk in only one of the six animals which failed to respond to light. As no trace of it was found on the serial sections, it had presumably been displaced from the operative site to the nasopharynx shortly after the operation.

In three of the ten animals which responded to light a wedge of scar tissue separated the pars distalis from the median eminence, and there was no direct vascular connection between the two (by which is meant there were no vessels actually connecting the two structures). Such vessels were present in five of the remaining eight.

The ninth and tenth animals which responded provide an even more important and critical test of the hypothesis that is under examination. In one the operation had disrupted the floor of the third ventricle, starting just behind the optic chiasma, and the whole pituitary portal system of vessels, as well as the pars tuberalis, which was represented by only a few scattered basophil cells, had been ablated. Median eminence and pituitary stalk could not be recognized. The prominent sinusoids of the pars distalis were supplied mainly by vessels from, and drained mainly into vascular channels in the connective tissue surrounding the gland. In addition, they communicated in the midline with what appeared to be venous channels in the disorganized tissue which formed the prolapsed part of the floor of the third ventricle.

The last animal was even more decisive, and a glass-plate reconstruction was made of the serial sections of the specimen in order to determine the final shape and position of the paper that had been placed between the divided ends of the stalk. This reconstruction, and the serial sections, show that the paper had become completely surrounded by reaction tissue,

(b) Observations which add up to the same general conclusion are reported by Tang and Patton (1951), who studied the effects of stalk section in the male guinea-pig. Histological study revealed "little, if any, direct (vascular) connection between the hypothalamus and the hypophysis". It was nevertheless found that unless the pars distalis suffers from serious infarction, its growth-promoting, ACTH and gonad stimulating properties are unimpaired.

(c) Studies made in my own laboratory have also provided clear-cut results that are in keeping with these views. Mature female ferrets were used in these experiments, which were designed to discover whether division of the pituitary stalk prevents these animals from coming into heat when exposed to additional light during the winter anœstrum. This response is mediated through the retina and optic nerves, and does not occur if the pituitary is removed. The general belief is that some part of the hypothalamus is a relay station in the pathway taken by the stimulus on its way to the pars distalis.

The published reports of our own findings relate particularly to 16 ferrets (out of a much larger number operated on) in which the stalk had been completely severed during the summer of 1951 (Thomson and Zuckerman, 1954). In four animals muscle, and in four others waxed paper, was placed between the proximal and distal parts of the divided stalk. Artificial lighting was begun on October 25, 1951, and was kept on daily for six hours, between 4.30 p.m. and 10.30 p.m. Normal control animals were kept both under ordinary conditions of lighting and in the room which was kept illuminated. The end of the experimental period was set by the day during the spring of 1952 when all 19 unoperated ferrets in the control and unilluminated room were in heat. At this time six of the 16 animals in which the pituitary stalk had been successfully divided were still anœstrous, and by definition they had, therefore, failed to respond to the artificial light. In three of these six the proximal stump of the stalk was adherent to the pars distalis (as determined from a study of serial sections of the region), and there was a clear anastomosis

It was found that the whole of the sella turcica had been roofed over by a massive barrier, which considerably exceeded the pituitary gland in size, and which consisted of a central avascular core of broken-up cellulose surrounded on both sides by practically continuous new bone, which in turn was covered both above and below by fibrous reaction tissue containing vessels. The margins of the barrier were continuous with the connective tissue (dura) lining the floor of the skull and with the arachnoid membrane. In order to bring out these points clearly, a wax plate reconstruction of the whole *region was made from the serial sections* by the usual *methods* used in embryological studies.

Fig. 1 is a photograph in perspective of the base of the brain with the barrier (painted black) and pituitary gland (P) in place. Fig. 2 is a photograph of the under-surface of the floor of the brain, which demarcates (a) the complete limits of the *median eminence (ME) containing the primary capillary network* of the hypophysial portal system, and the remains of the proximal stump of the pituitary stalk; and (b) the basilar artery and its terminal branches (B). Fig. 3 is a lateral view of the barrier with the outline of the pars distalis (P) (the neural process having completely disappeared) in position. Fig. 4 shows the under-surface of the base of the brain with the barrier and pituitary gland (P) in position. It will be seen that the pituitary gland was nowhere in contact with the base of the brain (the relative position of the median eminence (ME) has been projected on to the lower surface of the barrier).

These points are further illustrated in Figs. 5-11. Fig. 5 is a low-power photomicrograph of a median sagittal section of the specimen. It shows the pars distalis (P) with the intercavernous sinus behind it, overlaid by a barrier consisting of a central core of broken-up cellulose (illustrated, below the layer of bone, in high-power view in Fig. 14), enclosed above and below by a plate of new bone, on the lower and upper surface of which is fibrous reaction tissue. Fig. 6 shows the disposition of the barrier at the extreme right-hand margin of the median eminence and proximal stump, indicated

in which platelets of new bone had developed, presumably from bone dust introduced at the operation by the drill. The remains of the proximal stump of the stalk, containing vessels, and the median eminence, containing the primary capillary loops, were adherent to the scar tissue on the upper surface of the paper, while the degenerated remains of the distal stump were closely applied to the reaction tissue on its under-surface.

All the vessels of the proximal stump and of the median eminence drained into the upper layer of fibrous tissue, and close study of the serial sections failed to reveal any kind of vascular connection between the pars distalis on the one hand, and the median eminence or adjacent parts of the hypothalamus on the other. On the other hand, it provided every anatomical reason to suppose that the blood which flowed in the capillaries and venules in the reaction tissue forming the upper surface of the barrier drained immediately into veins and venous sinuses in the tissues by which it was surrounded, and so into the systemic circulation.

As already observed, the set of experiments was carried out in the winter of 1951. They have since been repeated, and their results confirmed. But only one preparation (T. F. 836) will be mentioned here, as it, too, provides a critical test of the hypothesis. The pituitary stalk of this animal was divided on 15 October, 1953, and a platelet of waxed paper was inserted between the proximal and distal ends of the stalk. Exposure to artificial light began some two weeks later, and the animal started coming into heat thirty-six days afterwards and was in full heat in fifty-one days. The corresponding figures for 14 unoperated controls kept under the same conditions were thirty-three to fifty-eight and fifty-seven to eighty-three days. The animal was then killed, and after its vascular system had been perfused with Indian ink, the usual histological preparations were made of the tissues. Serial sections were made of a block of undisturbed tissue comprising the hypothalamic region of the brain and the adjacent part of the base of the skull.









(For explanation of figures see overleaf)

by the letter A. A high-power view of the extreme right lateral margin of the stump is shown in Fig. 12. Figs. 7 and 13 are corresponding views of the left-hand margin of the median eminence and proximal stump of the stalk, indicated by the letter B. From side to side the median eminence and proximal stump measure 1.15 mm. New bone, encasing its core of cellulose, extended further laterally on both sides, but more so on the right than on the left. This is illustrated by the planes of section shown in Figs. 8-11. Fig. 8 shows the sagittal plane of a section 0.7 mm. to the right of the extreme lateral margin of the median eminence, and Fig. 9, 0.95 mm. to the left. In both cases the barrier still consists of cellulose, bone and fibrous tissue. The corresponding Figs. for 0.1 mm. further out to the left and right are shown in Figs. 10 and 11. Fig. 11 shows that where bone and cellulose disappeared on the left side, the upper surface of the pars distalis was separated from the base of the brain (here well lateral to the median eminence) by an intact pia-arachnoid membrane, and by a thinnish layer of reaction tissue into which cells of the pars distalis had extended as an adenomatous leaf-like growth 20μ thick. On the lateral side of this adenomatous leaf, the fibrous tissue again thickened, before thinning out again over the lateral 0.2 mm. of the pars distalis. Behind the gland the barrier still remained thick and densely fibrous, and still showed small islets of new bone. On the right-hand side the upper caudal part of the pars distalis extended backwards as a thin tongue, which is illustrated in Fig. 10, marked A. On this side the barrier consisted of a thick and dense fibrous tissue almost to the lateral limit of the pars distalis, about 0.2 mm. lateral to the plane of section illustrated in Fig. 10. The pia-arachnoid was also intact on this side.

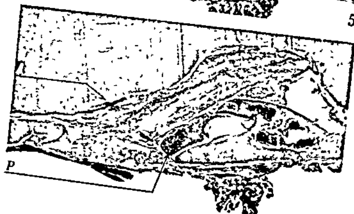
All the vessels in the median eminence and in the remains of the proximal stump of the stalk drained into vascular channels in the fibrous tissue on the upper surface of the barrier. This is illustrated in Fig. 14 and by Figs. 12 and 13, which show the lateral limits of the median eminence and proximal stump. The closest examination of the sections failed to reveal any



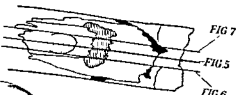
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6



FIGS. 5, 6 and 7. Low-power photomicrographs of sagittal sections through sella turcica and base of brain through planes indicated

Fig. 1 Wax plate reconstruction of serial sections of TF. 836, a ferret which responded to light after the division of its pituitary stalk. P = pituitary. × 9 5

Fig. 2. Lower surface of reconstruction of base of brain showing median eminence ME and basilar artery B. × 9 5

Fig. 3 Lateral view of barrier of reaction tissue separating pituitary gland P from base of brain × 9 5

Fig. 4. View of pituitary and base of brain with barrier of reaction tissue in position. The outlines of the median eminence have been projected on to the under-surface of the barrier. × 9 5



3







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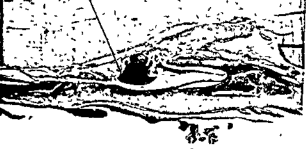
Fig. 4 View of pituitary and base of brain with barrier of reaction tissue in position. The outlines of the median eminence have been projected on to the under-surface of the barrier × 9 5

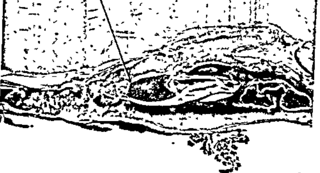


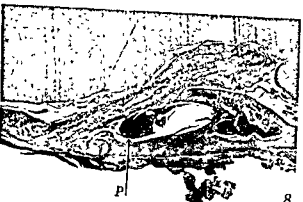


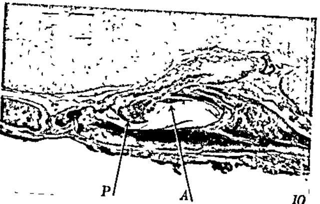


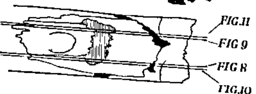
(For explanation of figures see overleaf)











Fics 8, 9, 10 and 11 Low-power photomicrographs of sagittal sections through sella turcica and base of brain through planes indicated in key × 11 3







(For explanation of figures see overleaf)

vessels in the median eminence draining into collecting channels other than those which immediately entered the fibrous tissue over the central part of the barrier, to which the eminence was directly applied.

In so far as they could be identified, the afferent vessels to the sinusoids of the pars distalis, which drained into the surrounding venous sinuses, were derived from neighbouring arterioles below the barrier. A few minute vessels in the 0.02 mm. leaf of adenomatous tissue which extended upwards from the left-hand upper surface of the pars distalis could not be identified as either afferent or efferent.

The ovaries, adrenal cortex and thyroid gland of this animal, as of all others whose pituitary stalks had been divided and which became œstrous, were normal in size and histological appearance. In those which remained anœstrous,

Discussion

As I have already remarked, all the reports on pituitary grafts that have been cited relate to experiments in which care was taken that the animals into which the implants were made had been completely hypophysectomized; and with the exception of some of Cutuly's experiments, all refer to grafts in sites remote from the median eminence and hypothalamus. Similarly, in the interpretation of their experiments on stalk section, both Greep and Barrnett and Tang and Patton state, on the basis of studies of serial sections, that no direct vascular link had re-established itself in their animals between the median eminence and the pars distalis. Making sure of this point was also a major concern in the experiments on ferrets carried out by Thomson and myself.

Bearing this in mind, the experiments to which I have referred indicate that normal responses of the pituitary can occur after transplantation or after stalk section. Some of these responses (e.g., the response to light, the recurrence of

œstrous cycles) are among those in which it is assumed that the hypothalamus plays the part of a relay station. It therefore follows that the answer to the question to which I have addressed myself in this paper is that a direct vascular connection between the base of the brain and the pars distalis is neither sufficient, other things being equal, nor necessary for positive responses of the pars distalis. The proof that it is not sufficient is that in cases where it has been present, responses have failed to occur. More decisively, the proof that it is not necessary is that responses can occur in the complete absence of any direct vascular connection. For these reasons it is necessary to reject the hypothesis that the pituitary portal vessels are an essential pathway in a process whereby the hypothalamus "controls" various functions of the pars distalis.

In the course of our own work on the ferret we have made many negative observations of the kind which form the background to the view that has now been shown to be invalid. These "failures" also indicate that the transplanted pituitary gland, or one whose stalk has merely been divided or manipulated surgically, ceases to function properly, and may cease to function at all. Although one single positive observation counterbalances any number of observations of hypofunction or non-function in the kind of test with which we are concerned, it is nevertheless necessary that these negative observations should, if possible, be explained.

All seem to point to the general view that the degree of tissue differentiation represented by the pars distalis is so great that the gland is very quickly damaged by any alteration in its physical environment. What seems to be an excellent illustration of this sensitivity is given by Harris (1950) himself,* when he noted that signs of pituitary dysfunction may occur if the pituitary stalk is just manipulated, let alone cut. Clearly the most probable explanation for an

*He notes that "the pituitary was exposed in fifteen female rats, and in some was manipulated, but not cut. A pause or irregularity of the œstrous cycles occurred in some cases, but all animals eventually (*sic*) returned to a regular œstrous rhythm".

observation such as this is that the manipulation disturbed, or even tore, vessels in the pituitary stalk. Since these vessels play so important a part in the supply of the sinusoids of the pars distalis, it is not at all surprising that subsequent pituitary dysfunction occurred. Harris (1950) has also pointed out—what his detailed descriptions illustrate even more clearly—that atrophy and necrosis of the pars distalis frequently occur after stalk section, and that they might be due to a diminution in the total blood supply to the gland. He takes the view, however, that "reduction in the amount of anterior lobe tissue" as a result of what he calls "diminished blood supply" is unlikely to have been responsible for post-operative disturbances in the reproductive cycles of his stalk-divided rats, since "in many cases the normality or otherwise of the reproductive processes bore no relation to the amount of anterior lobe tissue present".

In our own studies of the ferret we have also tried to differentiate responding from non-responding animals by reference to the size and structure of the pars distalis. Our experience is that it is quite impossible to predict from the animal's response whether the size or histological appearance of its pituitary would be normal or abnormal. This is partly illustrated by Figs. 15 and 16. Fig. 15 shows the appearance of the pituitary cells in the ferret illustrated in Figs. 1-14, and which reacted to light, in spite of the lack of a vascular link between its median eminence and pars distalis. Fig. 16, taken at the same magnification, is of an animal which had had its stalk divided, and which had failed to respond to light, even though there was a clear vascular connection between the pars distalis and the proximal stump of the pituitary stalk. So far as responses are concerned, the figures might have been reversed.

Greep and Barrnett have suggested that the functional capacity of a pars distalis after division of the pituitary stalk is likely to be dependent on the extent of the infarction which it has suffered, and on the rate and degree of revascularization. There is, however, no way of which I am aware of correlating

the cytological appearance of such damaged anterior pituitary glands with their functional capacity, or of determining in what circumstances critical and adverse changes may be caused. The immediate disposition of the blood vessels in different specimens varies from individual to individual, as becomes abundantly clear when one compares the recent studies on the human pituitary made by McConnell with those made by Xuereb *et al.* This variability may, indeed, be far greater than is suggested by their actual descriptions. For example, it seems that in some circumstances the stalk may be practically an avascular structure, in spite of the fact that the straightforward anatomical indications are that all or part of the blood to the sinusoids of the pars distalis flows through the pituitary portal vessels. Thus, Dandy (1940), in describing an operation on the optic tracts of a young woman aged 17, has recorded that, "the hypophysial stalk was divided with scissors midway between the base of the brain and the diaphragm of the sella. This was done without the slightest bleeding, and without sponging either to the cut side of the stalk or to the contiguous region. The stalk was about 1 cm. long". Professor Sir Geoffrey Jefferson, whose opinion I sought on this matter, tells me that when pituitary adenomas are removed much more free bleeding occurs than could possibly be accounted for by the stalk vessels. In cases where the pituitary stalk has been deliberately cut (as in hypophysectomy for breast cancer) there is considerable bleeding from the gland which seems to come from branches of the carotid vessels. Greep and Barrnett (1951) have also noted that hæmorrhage may be insignificant when the pituitary stalk of rats is cut; and the same is frequently true when the pituitary stalk of ferrets is divided. All this indicates that it is impossible to predict the detailed vascular anatomy of any individual pituitary from a general description of the blood supply of the gland; and consequently that the amount of vascular damage and subsequent functional damage which the pituitary gland will suffer after stalk section is both variable and unpredictable.

This view is contrary to Harris' belief that a one:one relation exists between "pituitary function and pituitary portal vessels", and "no function and no pituitary portal vessels". But the facts that he himself reports (1950) in his main paper on the division of the pituitary stalk hardly lend themselves to so categorical a view. For example, he describes a central core of necrosis, which was "of marked degree in eight", in the pars distalis in 20 of 23 adult female rats whose pituitary stalks had only been divided (no foreign body was placed between its cut ends). He also notes that "some vascular connexion between the median eminence and pars distalis had been re-established in every case". Yet one of the animals concerned did not manifest an œstrous smear at any time in the one hundred and fifteen days after the operation, and in eight animals the phases of œstrus that did occur were irregular, and separated from each other by as many as fifty days. A vascular connection between the median eminence and the pars distalis was also present in two of four rats which remained anœstrous after their pituitary stalks had been divided, and the two ends separated by means of a pledgelet of cotton-wool. Similarly, 11 of 19 rats in which a small plate of paper was placed between the two ends of the pituitary stalk, are reported as having remained anœstrous for periods varying from fifty-eight to one hundred and six days, when they were killed. In five of these the pars distalis showed marked central atrophy, and in five moderate atrophy. No mention is made of the portal vessels in these 11 animals, but in seven others subjected to the same operation, the cycles which occurred after the operation are described as having been irregular, even though in five of the seven the median eminence was connected with the pars distalis by "a fairly rich plexus of capillaries".

Harris obviously obtained the impression that the extent of the vascular connection between the median eminence and the pars distalis was correlated with the extent to which reproductive functions became normal after stalk-section. But the facts on which this conclusion is based are not available in his

paper. And so far as the action of possible hypothalamic chemotransmitters is concerned, it is difficult to see why their assumed effect on the pars distalis should be proportional to the precise number of vascular channels, given that at least some are present, connecting the capillary plexus in the median eminence with the gland.

An examination of the results of stalk section reported by Westman and Jacobsohn (1938*a*, *b*, *c*, *d*, *e* and *f*) makes it plain that the functional effects of the operation varied considerably from individual to individual in their experiments, and also that some of their results differ from those of recent workers. Full histological details of the site of lesion are not always given, but the usual procedure followed by these two workers was to divide the stalk, and to separate its two ends with a small platelet of silver paper. The published accounts of the experiments often note that serial sections were made in order to see whether the stalk had been successfully divided. But while the reports indicate that the operations were often unsuccessful, they nowhere indicate whether, in those instances where the division was complete, the platelet of silver paper had prevented a vascular link from re-establishing itself between the base of the brain and the pars distalis.

The main experiments of Westman and his colleagues were carried out in the period before Harris had emphasized the proviso that the physiological results of pituitary stalk section cannot be properly evaluated unless it is known whether the pituitary portal vessels had remained uninterrupted or had regenerated. Those which have recently been reported by Benoit and Assenmacher (1951*a* and *b*, 1952, 1953*a* and *b*), and by Assenmacher and Benoit (1953*a* and *b*), were made in the full light of this consideration. These two investigators have studied the growth which occurs in the testis of drakes when the birds are kept under conditions of artificial illumination in the autumn and winter months—a response analogous to that of the ferret which I have already described.

In the first of their papers (1951*a*) bearing on the problem

under review, these two workers note that the hypophysial portal vessels are the main afferent supply to the sinusoids of the pars distalis of the duck, and that at the same time the pars distalis receives an inconstant but small number of branches from the inferior hypophysial arteries. One to three *arterioles are also described as entering the gland directly from* the superior hypophysial arteries. The portal veins which drain the capillary plexus in the pars tuberalis, and which are held to be the main supply of the sinusoids in the pars distalis, vary up to about two dozen in number, and form a bundle of vessels which lies about 2 mm. in front of the pituitary stalk itself. In a later paper (1953*b*) the bundle is described as being 200μ in one diameter; 150μ in the other; and from 250μ to 500μ *long*.

In their next paper on the subject, Benoit and Assenmacher (1951*b*) reported that numerous thin nerves emerge from the tractus hypophysius and loop towards the capillary plexus in the pars tuberalis before continuing their course on the surface of the tractus into the neural process. These fine nerve loops are said to be rich in a substance which reacts to Gomori's chrome alum-hæmatoxylin-phloxine stain. In a paper published in 1953(*b*), they consider, without coming to any new conclusions, whether these fibres are axons from the same hypothalamic nuclei from which the main tractus hypophysius is derived.

In further notes Benoit and Assenmacher (1952) and Assenmacher and Benoit (1953*a* and *b*) reported that lesions which interrupted the tractus hypophysius "high" in the median eminence led to complete atrophy of the neural process. This experiment was performed on four ducks, and was also associated with degenerative changes in the pars distalis, and involution of the testes. It is stated that the bundle of portal veins was intact in these four birds. In two other birds, with superficial lesions in the median eminence so placed *that the portal vessels were damaged, the pars distalis* is said to have been more or less normal, and the testes fully functional.

paper. And so far as the action of possible hypothalamic chemotransmitters is concerned, it is difficult to see why their assumed effect on the pars distalis should be proportional to the precise number of vascular channels, given that at least some are present, connecting the capillary plexus in the median eminence with the gland.

An examination of the results of stalk section reported by Westman and Jacobsohn (1938*a*, *b*, *c*, *d*, *e* and *f*) makes it plain that the functional effects of the operation varied considerably from individual to individual in their experiments, and also that some of their results differ from those of recent workers. Full histological details of the site of lesion are not always given, but the usual procedure followed by these two workers was to divide the stalk, and to separate its two ends with a small platelet of silver paper. The published accounts of the experiments often note that serial sections were made in order to see whether the stalk had been successfully divided. But while the reports indicate that the operations were often unsuccessful, they nowhere indicate whether, in those instances where the division was complete, the platelet of silver paper had prevented a vascular link from re-establishing itself between the base of the brain and the pars distalis.

The main experiments of Westman and his colleagues were carried out in the period before Harris had emphasized the proviso that the physiological results of pituitary stalk section cannot be properly evaluated unless it is known whether the pituitary portal vessels had remained uninterrupted or had regenerated. Those which have recently been reported by Benoit and Assenmacher (1951*a* and *b*, 1952, 1953*a* and *b*), and by Assenmacher and Benoit (1953*a* and *b*), were made in the full light of this consideration. These two investigators have studied the growth which occurs in the testis of drakes when the birds are kept under conditions of artificial illumination in the autumn and winter months—a response analogous to that of the ferret which I have already described.

In the first of their papers (1951*a*) bearing on the problem

posterior portal veins, in an area of median eminence which is no more than about a millimetre across, and on the suggestion that the former alone arise from a region that is rich in Gomori-positive material. But since the division of the portal vessels was so rarely complete (sometimes only the most anterior were divided), the alternative explanation, that the positive and negative responses observed by these two workers could be related to the degree of vascular damage suffered by the pars distalis, seems equally valid. For we are not dealing just with one lot of birds which responded and another which did not, and which differed from each other only in the number of divided portal vessels. In both groups variable amounts of damage had also occurred in the pituitary. Negative observations can no more provide the answer here than they did in the case of the ferret.

Other than the function which has been discussed in the present paper, it is difficult to see what special significance can, or should be, attached to the pituitary portal vessels. Observations reported by Greep (1936) and Cutuly (1941) suggest that pituitary grafts "take" far better in the sella turcica than elsewhere. In Greep's experiments about 70 per cent of "takes" occurred in 32 rats. These animals underwent typical œstrous cycles, and 13 pregnancies were recorded. On the other hand, Harris and Jacobsohn (1952) found that 12 of 14 rats bearing grafts in the pituitary capsule (i.e. sella turcica) failed to undergo any œstrous cycles, whereas they occurred in five of ten rats with grafts under the median eminence. Harris's interpretation of this observation is that the implanted tissue possibly became vascularized by vessels arising from the primary plexus of the hypophysial portal system. Further experiment is clearly necessary in order to discover why grafts into the sella turcica succeeded in the hands of Greep and of Cutuly, but failed in those of Harris and Jacobsohn, before this interpretation of the success of the grafts made under the median eminence is finally accepted—particularly since other observations also show that a completely functional pars distalis can exist in the sella turcica

From these experiments, Benoit and Assenmacher drew the conclusion that lesions which interrupted the tractus hypophysius deprived the capillary vessels in the median eminence of some stimulation previously mediated by the nerve loops which they had previously described Hence hypofunction of the pituitary gland occurred. The visible sign of this stimulation is the Gomori-positive substance found in the part of the median eminence from which the portal vessels spring.

The next step (Assenmacher and Benoit, 1953b) was to divide the bundle of portal veins (tractus porto-tubéral) independently of the pituitary stalk itself. The operation was done on four birds, and in some of them (number not stated) a thin plate of sclerotic was placed between the proximal and distal stumps of the bundle of vessels. These animals did not respond to artificial light. Post-mortem examination showed that the "tractus porto-tubéral" had been completely interrupted, and that the region of the lesion was occupied by a series of inter-communicating vascular pockets which also opened into the cavernous sinuses. It is stated that no portal channels were re-established between the median eminence and the pars distalis, but that some portal vessels had not been divided, and that they linked the posterior part of the median eminence, which is said not to contain Gomori-positive substance, with the pars distalis. In four other ducks the division of the bundle of vessels was incomplete or, alternatively, regeneration had occurred, as a large number of anterior portal veins linked the median eminence with the pars distalis. These four animals reacted to light and had testes of normal size.

All these observations, together with a few others, have been assembled in two general papers (Benoit and Assenmacher, 1953a and b). One can either interpret them in the way Benoit and Assenmacher suggest, or consider the results of the experiments in which the bundle of portal vessels was divided as dependent on the degree of vascular damage suffered by the pars distalis. Benoit and Assenmacher's interpretation hinges on the differentiation of anterior from

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without any direct vascular connection with the median eminence.

The experiments discussed in this paper also make it clear that a pars distalis can function normally, and in a "reflex" manner—if the light response of the ferret can be so described —without any nerves connecting it with the hypothalamus. On the other hand, Abrams, Marshall and Thomson (1954) have now found that the "nervous" control of pituitary function is more complicated than is indicated by just a consideration of the connections of the pars distalis with the hypothalamus—for they have shown, in experiments on seven female ferrets, that bilateral superior sympathetic ganglionectomy consistently inhibits the response to light. And the whole problem is further complicated by a possible functional relation between the anterior and posterior lobes of the pituitary (see Zuckerman, 1954). Several recent papers suggest that there may be a relation between the release of antidiuretic hormone and that of ACTH (e.g., Mirsky, Paulisch and Stein, 1954), and Itoh and Arimura (1954) go so far as to suggest that posterior lobe hormone may inhibit the release of ACTH from the pars distalis. If the pituitary portal vessels can no longer be regarded as an elective pathway by which the functions of the pars distalis are controlled in some mysterious way, the possibility still remains, therefore, that the activity of the anterior pituitary may be integrated with that of the neurohypophysis—and so with the hypothalamus.

Acknowledgement

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DISCUSSION

HARRIS. I should like to say how much I enjoyed Professor Zuckerman's philosophy, but if I may say so, I don't think his attitude to the literature was very critical. For example, Schweizer and his colleagues in some of their studies (Schweizer, M, Charipper, H. A. and Kleinberg, W., 1940, *Endocrinology*, 26, 979) examined the completeness of their hypophysectomy by scraping the sella turcica and sectioning questionable fragments. May (1935, *C. R. Soc. Biol., Paris*,

120, 867; and 1937, *C. R. Soc. Biol., Paris*, 124, 920) grafted pituitary *tissue into the anterior chambers of the eyes of four rats (two male and* two f

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by ocular pituitary transplants, as compared with hypophysectomized controls, but the glands were far from normal in size. This has been observed by other workers—that anterior pituitary transplants, in a

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of its normal activity

field in the pituitary region so that histological studies of the region may be made more easily at a later date.

The pituitary stalk of 24 ferrets has been exposed by this method. In four animals the stalk was left intact, in six simple stalk

animals, their vascular system was perfused with 1:3 Indian ink and

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DISCUSSION

HARRIS: I should like to say how much I enjoyed Professor Zuckerman's philosophy, *but if I may say so, I don't think his attitude* [illegible] sectioning questionable fragments. May (1935, C. R. Soc. Biol., Paris,

the pituitary region of one of their critical ferrets Inspection revealed that a significant portion of the visible blood vessels did not contain Indian ink. Conclusions drawn from incompletely injected specimens must be regarded as unreliable.

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competent and concerned not to mislead their readers in what they wrote. I shall restrict my remarks to the four points Professor Harris made about our own work.

The first concerns numbers. In these experiments I am not impressed by numbers alone, any more than I was by the comparison of averages in the experiments on thyroid activity, where Professor Harris himself

and that in these animals few, if any, vascular connections had re-established themselves between the median eminence and the anterior

derive directly from Professor Harris's thesis that the anterior pituitary

In order to test the first proposition, Dr. Thomson and I have tried, over a period of more than six years, to obtain one or more preparations in which there is an absolutely effective barrier between the pars distalis and the overlying hypothalamus, and where the animal concerned responded to light This we have finally achieved. If we had achieved it only once, it would effectively have disposed of the first proposition which we set out to test

The second proposition, that the observation of normal pituitary function implies that the pituitary portal vessels drain directly into the anterior pituitary, has also been shown to be invalid, since we now have

serial sections eventually cut at 160 μ, following celloidin imbedding, through blocks of tissue containing the hypothalamus, pituitary gland and base of the sk[illegible]" Examination of this material showed: (*a*) The *pituitary stalk had* [illegible] *tioned in all but the blank-operated controls* [illegible] correlated with the presence o[illegible] of the tuber *cinereum* [illegible] tions were clearly vision[illegible] œstrous. Study of three animals in this grou[illegible] inserted, showed that the plates had been misplaced (two we[illegible] too anteriorly, and one obliquely) and that vascular connections had been re-established around the borders of the plates. No case of œstrus was observed in the absence of vascular connections. (*c*) The anœstrous animals had few, if any, vascular connections between the median eminence and anterior pituitary gland. The animals in this group (one simple section, and nine with plates) showed a well marked primary plexus and a well vascularized adenohypophysis. (*d*) Some atrophy of the pituitary gland occurred in all animals in which the stalk was sectioned. Measurement of the total volumes of the pituitary glands, and of the volumes of the two lobes by the "paper weight" method revealed that the average volume of the anterior lobe of the œstrous ferrets was 80 per cent that of the controls, and for the anœstrous group was 95 per cent of the controls. It is clear that, for the ferret at least, the suggestion of Greep and Barrnett (1951) that reproductive abnormality *following stalk section is due to atrophy of the* anterior lobe of the pituitary, is not true.

The conclusion we drew from these results is that the hypophysial portal vessels form a necessary part of the pathway of the light-induced œstrous response of the ferret

In considering the results of Thomson and Zuckerman in the light of our own work, we feel the following points are of importance: (i) Their conclusions are based on results in very few animals. It is true that positive results are more valuable than negative in experiments of this sort, but in drawing conclusions from such a small group of animals it becomes important to rule out the possibility that faulty techniques have failed to demonstrate vascular connections that do, in fact, exist.

(ii) The operative approach—the buccal route—used by these workers gives poor facility for placing plates between the cut ends of the stalk, and as they say the plates are "with a few exceptions.. eliminated from the wound" Also this operation often results in anatomical disorganization of the pituitary stalk region which complicates the final histological study.

(iii) The microscopic study of serial sections through the pituitary-hypothalamic region was made after embedding the specimens in wax and sectioning at 10μ (Zuckerman, personal communication). In our experience it is necessary to imbed in celloidin and cut thick sections at 100–200μ in order to obtain a clear picture of the vascular anatomy.

And lastly, (iv) through the kindness of Dr. Thomson and Professor Zuckerman, we have had the opportunity of examining sections through

One last observation needs to be made. We do not know why the pituitary glands of some animals respond and those of others do not, after section of the pituitary stalk. What our observations show is that

Deltour. To return to Prof. Harris's paper, I was amazed at the quantity of thyroxine he used in his thyroid experiments, considering that the turn-over rate in the rabbit must be around 5-7 μg. a day.

any deiodination. The extracts were then taken in acid and alkaline butanol and run on paper chromatograms. By the autoradiogram technique we were able to show that hypophysectomy does not change the ratio between the iodinated thyronines (thyroxine and triiodothyronine) and the iodinated tyrosines (monoiodo- and diiodotyrosine) but only decreases the rate of their biosynthesis (Roche, Deltour, Michel and Valez, 1953, *C. R. Soc. Biol., Paris*, **147**, 270).

Harris: In our rabbits the rate of secretion of thyroid hormone is about the equivalent of 15–18μg. of thyroxine per day. Dr K. Brown-Grant has a paper in press on this point. In view of this I agree that

twenty-four hours to different temperatures. They were killed after the

several ferrets—additional to those in which there was a barrier between the hypothalamus and pituitary—which have responded to light when no direct vascular connection can be demonstrated between the pars distalis and the remains of the proximal stump of the pituitary stalk containing the primary capillary net.

Professor Harris's second point of criticism concerns our operative approach to the pituitary stalk. I do not know what his experience is of the approach we have used, and I am, therefore, unable to say whether

we can obtain, by any means whatever, a preparation in which the

they get to the top, they will still see the same view.

Professor Harris's third point concerns the thickness of our sections. Here our experience simply does not accord with Professor Harris's. 10μ sections adequately demonstrate such vascular connections as exist between the pars distalis and the tissues by which it is surrounded. It just so happens that in our critical preparations these connections are present in all places except where Professor Harris's thesis demands that they should be. This is evident from the slides which I have shown*.

Furthermore, I think that Professor Harris's remark here implies that he has not quite grasped the significance of our preparations. In what he calls our critical preparations we have been concerned to show whether the primary capillary net of the pituitary portal vessels is present, and if

tissue†. In both this impervious barrier happens to satisfy, completely, the anatomical requirements of our operation—and the two animals in question responded to light.

This brings me to Professor Harris's last point. Had Professor Harris

serial sections, but weeks. Other anatomists have also examined our preparations for far longer than did Professor Harris or Mr. Donovan. Even if one could ever tell whether every minute vessel was full of ink—which I doubt—the suggestion that a significant proportion of the visible blood vessels did not contain Indian ink is quite incorrect. And to repeat, it is also irrelevant, since what matters is whether there was a

*Now illustrated in *Proc Roy Soc. B*, 1954, 142, 437.

† At the time of this discussion only one had been reconstituted.

operation.

Harris: The activities of the thyroid gland in the stalk-sectioned rabbits as compared with normals and hypophysectomized are now shown in Table I, p. 344

Zuckerman: Do the ranges just overlap?

Harris: No.

Thorn: We in this group are really indebted to Professor Harris and Professor Zuckerman for this discussion. It has been very helpful to have a problem as difficult as this reviewed by two very careful workers who are interested only in the absolute truth.

mechanism which Professor Harris has seen is the result of a two-fold mechanism in which he saw the TSH released, as we have seen in the intact rat, compensating for the effect seen in the thyroidectomized rat maintained with thyroxine. I think that is additional proof for these experiments of Professor Harris.

SOFFER: Professor Harris, have you any theory concerning the nature of the experimental inhibition of thyroid function following the administration of cortisone? In our experiments with the rat, ACTH or cortisone administered to the adrenalectomized or intact animal causes a reduction in the uptake of ^{131}I. The question arises as to whether this effect is the result of the inhibition of the elaboration of thyrotrophin or the

are capable of inhibiting the elaboration of TSH by the adenohypophysis.

HARRIS: We haven't done very many experiments on that line We

wonder whether the lymphopenia is necessarily a true reflection of adrenocortical activation. In other words, does adrenaline itself, in the

openia?

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pophysectomized animals have been killed. Twelve hypophysectomized animals have been tested with adrenaline, and in most of them we observed a negative response to this stimulus Of those which gave a positive response, the ^{131}I release slope which was done concurrently showed evidence of incompleteness of hypophysectomy. Only two animals which were ascertained to be completely hypophysectomized histologically have been tested for response to laparotomy, and the responses were *negative in both instances*.*

ZUCKERMAN: When I spoke before, I remarked on the reference to averages in Professor Harris's statement about thyroid responses, my

*These findings were *confirmed by the histological examination of additional* material.

During the three years which have elapsed since the last conference on this subject, the infant has accomplished a certain amount of growth. Above all there has been an encouraging tendency toward a working correlation of methods from such diverse sources as neurosurgery, neurophysiology, biochemistry, Gestalt psychology and psychoanalysis (Kubie, 1953; Heath, 1954; Ostow, 1954). Effective collaboration of workers from these different fields has depended on the degree to which methods were available for the observation of the integrated organism in action. This implies that the human adrenal as well as the human brain belongs to an organism of a certain complexity in pursuit of its needs for satisfaction and survival.

The complexity of the human organism from a neurological point of view refers to the various levels of integration as they were described by Hughlings, Jackson and Sherrington. Phylogenetically considered, the process of encephalization maintains the capacity to respond to stimuli with comparatively simple reflexes but also makes it possible for these responses to be integrated through higher levels of the nervous system. Grinker (1948) points out, for instance, that the hypothalamus, in which we are all so greatly interested these days, is not the only centre for autonomic activity, nor is it the most cephalad. As he states, "At each level of the nervous system such centers exist; that is, spinal, bulbar, pontine, etc. The hypothalamus represents visceral activity in more special, highly-organized combinations. Its orthosympathetic areas control and integrate all visceral activity; its parasympathetic areas are more highly specialized and discrete. However, autonomic representation (also) exists in the cerebral cortex, both of the parasympathetic and ortho-

antagonistic systems with cortical inhibitory influences usually predominating.

The analogy of the cortex and hypothalamus to the

PSYCHOLOGICAL RESPONSES TO THE ADMINISTRATION OF ACTH AND CORTISONE

Henry M. Fox, m.d.,
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At the Panel Discussion on "Psychophysiological Properties of the Adrenal Cortex" held at the meeting of the American Psychosomatic Society a year ago, the Chairman opened the session with a quotation from the "Address in Medicine" by William Gull, delivered in 1869, before the British Medical Association (Margolin, 1953). Gull, to whom Addison refers as his "former pupil" and "distinguished colleague" stated: "Modern physiology and pathology are advancing the evidence that whatever is living has nerve-quality in it. The highest expression of this quality is conscious intelligence. The lowest, it is from the nature of the case, at present impossible to mark. This much, however, it appears important to recognize clinically: that morbid brain force may give rise to a variety of disorders, apparently distinct from their original cause. There is a neuropathology from the brain to the tissues, as there is a reverse order of disturbances from the tissue to the brain." Eighty-two years later Professor Zuckerman (1952), who had presided at the Ciba Foundation Colloquium on "Hormones, Psychology and Behaviour", made the following closing remarks: "I therefore feel that while we have all gained a great deal of information from our various contributions, and that while we know a lot more than we did at the start about the possible articulation of endocrine factors with behavioural changes, we do not know how endocrine factors affect the nervous system or *vice versa*. That they do we can be certain; how they do is still a mystery. Our general concern with documentary matters indicates that both the clinicians and the experimentalists are dealing with a science that is still in its infancy."

observations in the different fields are made and conclusions are drawn.

Psychoanalysis pays particular attention to the psycho-

[illegible]

influences which have failed to mature with the rest of the personality. Psychoneurosis and especially psychosis are understood as forms of emotional regression to much earlier emotional patterns. This regression occurs in response to stress, which we may define as any external threat, any intensification of instinctual drives, or any increased demand from the conscience which challenges the capacity of the individual to maintain a balance between his needs and his satisfactions. This is, of course, analogous to the maintenance of the physiological balances referred to as homeostasis.

Pharmacological doses of ACTH and cortisone affect psychological as well as physiological balances In our study (Fox and Gifford, 1953) of over 100 patients who were given ACTH or cortisone for research study, for treatment of various diseases such as rheumatoid arthritis, asthma and multiple sclerosis, or for adrenal insufficiency (in Addison's disease or after partial or total bilateral adrenalectomy), the three most nearly constant findings and the three most regularly occurring together were alterations in appetite, sleep and motor activity. The range of psychological response has been found to include changes in intellectual alertness and mood as well as an increase or, more rarely, a loss in the capacity for warmth and friendliness in personal relationships. Some patients felt physically more alive and became more keenly aware of external reality and others were disturbed by feelings of unreality or of depersonalization. Although there was a wide variety of reaction, most of the patients who received therapeutic doses of ACTH or cortisone tended to experience one or more of the following subjective sensations: some feeling of increased well-being, heightened alertness and keenness of perception, an increase in appetite,

psychoanalytic structural concepts of the ego and id is evident. The hypothalamic influence on sleep, hunger, motor activity, energy and mood corresponds to the psychological representation of the instinctual drives (id). The activities of the cortex are, on the other hand, analogous to ego functions, such as the capacity of the individual to exercise judgment and to organize his impulses in accordance with comparatively long-term planning.

Freud, who was, of course, a distinguished neuropathologist before he entered the field of psychology, was well aware of this analogy. His respect for the complexities of mental life, including especially what he had discovered concerning the strength of repressed unconscious influences, led him to decide "to ignore the fact that the psychic apparatus concerned is known to us also as an anatomical preparation and carefully to avoid the temptation to determine the psychic locality in any anatomical sense. We shall remain", he stated, "on psychological grounds and we shall do no more than accept the invitation to think of the instrument which serves the psychic activities such as we think of a compound microscope, a photographic camera, or other apparatus. The psychic locality then corresponds to a place within such an apparatus in which one of the primmary phases of the image comes into existence." He goes on to remark that "we can give our conjectures free rein provided we keep our heads and do not mistake scaffolding for building." This was written by Freud in his epoch-making contribution, "The Interpretation of Dreams", which appeared in 1900. Recent advances represented, e.g., by the preoccupations of cybernetics or the complexities of reverberating brain circuits have stimulated some challenging speculations concerning the human brain but have certainly not reached the point where they can account adequately for the activities of the human mind. This makes it necessary for psychologists, neurophysiologists and biochemists who are engaged in collaborative research to respect each other's methods and to maintain a clear appreciation for the frame of reference within which

in response to large doses of cortisone and ACTH, and it could be shown that both young men were attracted to the experimental situation by an unusually intense need to prove their physical and emotional invulnerability. A few patients with neurotic symptoms of fatigue and unconscious fears of locomotion responded to ACTH and cortisone with great anxiety and increased inhibitions against movement. Two patients with anorexia nervosa experienced precisely the opposite of the expected "average reaction", refusing to eat altogether, vomiting, complaining of abdominal pain, and withdrawing into a state of mutism and complete immobility. These "negativistic" responses seemed to occur when the intensification of specific instinctual conflicts about eating and locomotion created intolerable anxiety and required an equivalent intensification in the neurotic inhibitions against these activities.

When the therapeutic effects of ACTH or cortisone brought about a sudden improvement in a long-standing disease process, certain patients developed unexpected complications. In some instances there was a recurrence or exacerbation of symptoms in spite of continued treatment with steroid hormones. In other patients new symptoms were elaborated which replaced previous ones. These included transient conversion symptoms in those patients who had a pre-existing hysterical character structure. A comparatively small number of patients developed more serious psychological difficulties, varying in extent from mild states of depersonalization to severe psychotic episodes, all of which were self-limited and relatively brief.

Every variety of clinical response to ACTH and cortisone seems to represent a summation of three principal components; each component plays a part in every reaction but its relative importance is determined by the personality structure of the individual patient. (1) the pharmacological effect of ACTH or cortisone on the total organism; (2) the meaning to the patient of an alteration in the symptoms of the disease for which he is being treated; and (3) the nature

a sense of increased energy for physical or intellectual activities, a renewed interest in their surroundings and former relationships and a greater capacity for emotional contact with others. When the administration of ACTH and cortisone was discontinued, most patients tended to experience a sense of emptiness and depression, a loss of appetite, a feeling of diminished physical and intellectual energy, a withdrawal of interest from their environment, and a feeling that emotional *contact with other people had been lessened.*

This sequence of changes in mood and sense of physical well-being tended to parallel changes in the symptoms for which the patient was receiving treatment. Energy seemed to increase with symptomatic improvement and diminish with the reappearance of symptoms when treatment was discontinued. The same changes were observed, however, in patients whose symptoms were unaltered by treatment with ACTH and cortisone, and certain patients who showed *marked symptomatic improvement failed to experience any* subjective changes whatever.

Certain patients experienced the sense of well-being in an exaggerated form, as if the increased instinctual energy was difficult to regulate within the limits of their customary defences. Some of these patients described their experiences as intensely pleasurable, in terms of great alertness, physical strength, or intellectual prowess, and their behaviour suggested a state of euphoria or elation sometimes approaching a mild hypomania.

Other patients became anxious, restless, wakeful, and complained of irritability, distractability, and racing thoughts. Apparently they perceived the physiological effects and the *increased instinctual drives* as threatening to their internal security. Sometimes these responses seemed to represent a general sense of alarm, as if the patient had become aware of a non-specific danger to his existing emotional equilibrium.

Patients who did not react in the usual way to the administration of ACTH and cortisone included two "normal control" subjects who denied any subjective sensations whatever

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of the patient's phantasies about the action of the substance he has been given.

Fourteen per cent of our patients developed psychotic-like disturbances in response to large doses of these drugs. Observation of these individuals gives certain clues concerning the physiological and psychological effects of these substances under less drastic circumstances. Our patients experienced a pathological disturbance of mood, of thinking, and of the perception of reality. Except for some equivocal disorientation in an elderly woman with dermatomyositis, none of our patients showed the clouding of consciousnesss, disorientation, or intellectual deficit that is characteristic of organic confusional states. Glaser (1953), reporting 12 psychotic reactions observed at the Presbyterian Hospital in New York, on the other hand, describes two major patterns: (a) a primarily affective disorder, either manic or depressive, and, more frequently (b) an organic (toxic) psychosis with associated paranoid hallucinatory and (or) affective (usually depressive) components similar to psychotic states occurring with Cushing's syndrome.

Different observers have, similarly, reported a correspondence or no correspondence between these psychotic states and disturbances in the electrolytes (particularly potassium depletion) or alterations in the electroencephalogram. The influence of ACTH and cortisone on existing psychoses has also been studied recently. There was no significant therapeutic effect in depressive or manic states (Cleghorn, *et al.*, 1950; Lehmann, *et al.*, 1950) or in schizophrenia (Glaser and Hoch, 1951). On the other hand, cortisone does have a beneficial effect in Addison's disease. It may not only ameliorate personality disturbances to a much greater extent than can be achieved by DCA treatment, but enables patients to experience a return of vigour far beyond the effect of DCA (Thorn, *et al.*, 1953). Cortisone also corrects abnormalities in the electroencephalogram. We have noticed, however, that in certain Addisonian patients severe psychological disturbances were apparently precipitated by

relatively small doses of cortisone and others have reported exacerbation of an existing psychosis and the precipitation of psychosis in response to cortisone (Cleghorn, 1952; Thorn, *et al.*, 1951).

There seems no reason to doubt the validity of these various types of observation even though some of them at first look as though they might be contradictory. Forces which elicit a functional response, after all, may also produce relatively irreversible structural changes with impairment and eventual destruction of cells and tissues. The fact that some individuals experience such profound psychological effects from taking ACTH or cortisone in the absence of any gross disturbances of electrolyte balances or of general toxic damage to brain tissue requires explanation in terms of more subtle biochemical influences on psychological processes—presumably more like the ordinary response of the organism to life stress.

We have recently attempted to differentiate life-long psychological patterns from emergency defences in patients undergoing cardiac surgery (Fox, Rizzo and Gifford, 1954). This helped to clarify the nature of relatively primitive reactions as they emerged under the prolonged and increasingly severe degrees of stress imposed on the individual by the progressive limitation of his activities because of advancing heart disease and finally by the threat of an operation which promised a cure but carried no guarantee of survival. This experience with patients who received neither ACTH nor cortisone provided a useful background with which to compare patients who responded to these drugs with a psychotic type of regression.

Even in patients less seriously affected, the ACTH or cortisone induced an increased responsiveness both to inner visceral sensations and to external sensory perceptions, while some of the patients who became anxious experienced an exaggerated sensitivity to light or noise. This apparently represented pathological distortion of an effect of the pituitary-adrenal hormones which seems to heighten perception,

perhaps as part of the readiness for action. Thus the quality of perception as well as the nature of behaviour and performance help to indicate the extent to which a useful sharpening of alertness has been exceeded.

In the Chairman's closing remarks at the last Ciba Foundation conference on this subject, Prof. Zuckerman (1952) emphasized that "it is not just some internal drive which is primed and 'fired' by differences in endocrine balance. As Mr. Eayrs emphasized in the second paper of the meeting, perception is also significantly affected by endocrine factors (and) this is a very important fact." Prof. Zuckerman went on to ask whether different intensities of motivation can be associated with changes in the significant features of a total stimulus pattern (the Gestalt). He reminded the group that their discussions seemed to have suggested "that specific neuromuscular patterns are less important than the total reorganization . . . of the activities of the organism."

One of our patients was a twenty-nine-year-old woman with lupus erythematosus which had begun after her marriage (Gifford, 1954) She was given 500 mg. of cortisone for twelve days. During the latter part of this period she developed a ravenous appetite with an increased intake of food. She was alert, wakeful, with increased sensory acuity and enhanced intellectual functioning. She then became more excited, could not sleep and was unable to concentrate. She became afraid of lying down at all, sleeping perhaps an hour or two during the night curled up in a chair. In her journal she noted at this time that "complete relaxation is death". Her ravenous appetite was now succeeded by an intense fear of eating. When food was brought to her she showed an immediate interest, her expression would become alert and she would eat a few bites eagerly. Then she would stop and refuse anything further, either food, fluids, or medication. When offered a glass of milk she refused it saying "that will make me turn into a baby" and she made the same remark when the nurse suggested that she curl up and make herself more comfortable. (Recalling this period after her recovery, she remembered

the specific fear that her *mother* was turning her into a baby.) Gradually she expressed more and more delusional distortions in the perception of her body, asking her doctors to examine her teeth and her knees because they were "fading away, in and out, appearing and disappearing". She experienced other changes in body image, feeling that her face was getting smaller, her legs had "faded away", that she had shrivelled up and become a baby, and that she was "dead already and brought back to life".

Werner (1940), the Gestalt psychologist, has compared the perception of children, of primitive man, and of psychotics. This patient's delusional belief that milk would turn her into a baby and her perception of her body as literally infantile illustrate Werner's description of psychotic regression. "Affect has once more become a factor in the configuration of the surrounding world as in the case of a genuinely primitive mind. And this occurs not only in the sense that the world of things becomes invested with an especially strong overtone of emotion, but rather in the sense that affect actually forms the world itself . . . The properties of things cease to be entirely objective, geometric and 'out there.' Actually they acquire and express a much greater 'depth' and inner significance. It is the very roots of expression which are bared by the pathological retrogression and the gradual replacement of a higher by a more primitive psychological level."

Aldous Huxley (1954) recently described his experiences after taking mescalin, whose effects have been compared to those of lysergic acid, pervitin, ACTH and cortisone (Hoch, 1951; Hoch, *et al.*, 1952; Glaser, 1953, Cleghorn, 1952; Rinkel, *et al.* 1952; Lindemann and Clarke, 1952)—all capable of producing what Hoch (1951) has characterized as "Psychotic manifestations in a state of clear consciousness". Huxley notes that he "spent several minutes—or was it several centuries?—not merely gazing at those bamboo legs [of the chair] but actually *being* them—or rather," as he explains, "being myself in them, or, to be still more accurate (for 'I' was not involved in the case, nor in a certain sense were 'they') being

my Not-self in the Not-self which was the chair." . . . "Visual impressions are greatly intensified and the eye recovers some of the perceptual innocence of childhood when the sensum was not immediately and automatically subordinated to the concept . . . These better things may be experienced (as I experienced them) 'out there' or 'in here,' or in both worlds, the inner and the outer, simultaneously or successively . . . in one respect at least, I was now a Not-self, simultaneously perceiving and being the Not-self of the things around me . . . Today the percept has swallowed up the concept." Huxley suggests that the effects of mescalin are the sort of effects you could expect to follow the administration of a drug having the power to impair the efficiency of the cerebral reducing valve. "When the brain runs out of sugar the undernourished ego grows weak, can't be bothered to undertake the necessary chores, and loses all interest in those spatial and temporal relationships which mean so much to an organism bent on getting on in the world."

Huxley, who made such accurate clinical observations, uses them to illustrate certain hypotheses concerning the artistic process and the nature of religious mysticism. Perceptual changes occurring under the influence of mescalin and in patients receiving ACTH and cortisone provide a special opportunity for the scientific investigation of a shift in the psychological balance of the individual from a relatively objective recognition of external reality toward a more subjective type of response. Psychic distance between the self and the outside objects shrinks and the boundary between private feelings and outer events becomes blurred. Primitivation of perception reflects the psychological regression from more highly organized and differentiated responses, to activity impelled to a greater extent by immediate instinctual drives and needs. It seems reasonable, moreover, to make the speculation that this disturbance in the balance of emotional drives and their psychological inhibition corresponds to some sort of short circuiting of cortical influence on hypothalamic impulses. Since the Gestalt psychologists have developed methods for

measuring the primitivation of perception, the qualitative changes can be quantitatively determined. These tests (e.g., Gottschaldt figures, which bring out changes toward more diffuse or more differentiated perception; solving metaphors, which tests emotional interference with abstract intellectual functioning; tests of sensori-motor co-ordination; and tests of certain autokinetic phenomena) can be given before, during, and after the administration of ACTH, cortisone or other drugs. Correlation of these findings with alterations in the patient's feelings and phantasies observed within the setting of a relationship to the psychiatrist during successive interviews, and concurrent observations of homeostatic balances as measured by appropriate biochemical indices, provide a promising multi-dimensional method for further investigation.

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DISCUSSION

DELTOUR: Would Dr. Fox like to comment on the experiments that have been done with adrenochrome, an oxidation product of adrenaline. It seems interesting to try to correlate these experiments with those which have been made with mescaline.

FOX: I only know what I have read in the *Journal of Mental Science*,* As you say, it is especially interesting because it may be a natural product. The kind of disturbances that were described are in line with what was found with mescaline, lysergic acid, etc There was not a toxic type of confusion but rather a severe disturbance in a state of clear consciousness For instance there were some rather severe depressions

[illegible]

was real suicidal danger. It is a fascinating opportunity for further research on the whole question.

DELTOUR: It would be important to know if the authors have used adrenochrome or the semicarbazone of adrenochrome since it has been claimed that adrenochrome has an ACTH-like action on the adrenal cortex. It is not easy to verify this statement because free adrenochrome is such an unstable compound, but the semicarbazone, which is very stable, shows this property to a certain extent, at least in animal experiments. The question arises whether adrenochrome can be considered in this case as acting directly, as an oxidation derivative of adrenaline, or whether it acts through the adrenal cortex

FORSHAM: Did these workers count the eosinophils in their work?

ZUCKERMAN. No.

ELMADJIAN. In that paper, the remark was made that the adrenochrome was unstable. If it was unstable it could not have been the semicarbazone, because that is very stable. The conjugated compound you are talking about is used as a styptic, isn't it? Some sort of antihæmorrhagic?

*HOFFER, A., OSMOND, H., and SMYTHIES, J. (1954). *J. ment. Sci.*, 100, 29.

DELTOUR: Yes, it is primarily a non-sympathomimetic hæmostatic agent, active on the bleeding time, but exerting no coagulating action.

ZUCKERMAN: I wonder whether the two normal undergraduates whom you used as controls, Dr. Fox, and who were out to show that ACTH

Fox: That is exactly what we have now embarked upon. We have

and after each of these different substances.

ELMADJIAN: It is very easy to get into difficulties in running experiments of this type. Two or three years ago Dr Malamud, Dr. Hope and

has any particular characteristics.

Fox: I can't give you a definite answer, but I would certainly agree that those individuals who respond with an increased appetite, as is also seen with the children, do have a certain specific configuration One has to consider what the intake of food means psychologically to different

*HOPE, T. M., ELMADJIAN, F and MALAMUD, W. (1951). *J. clin exp Psychopath.*, 12, 267.

people. One of the important aspects of primitivation is that people who *regress tend to look upon food as the equivalent to love or to affection.* We still retain that idea a little when we speak of someone as "honey".

BROWNE: One point struck me which you mentioned with regard to Aldous Huxley, in connection with Northrop's book on the Meeting of East and West—his description of feeling oneself into an object is emphasized very particularly by Northrop as characteristic of oriental art.

LUFT: I should just like to make a short comment about psychological changes after hypophysectomy. Our hypophysectomy patients are different types of people, and behave differently emotionally before the operation. After hypophysectomy, without any substitution therapy

read five.

THORN: We have a good deal of experience with work performance tests in Addisonian patients. Of course the difficult or impossible thing to control in the non-electrically stimulated work performance tests is motivation. And it is very impressive in these patients with Addison's disease who have 0 level of hormone to begin with, that with the first application of any quantity of cortisone, there is an intense capacity to do things, which is hard to visualize in terms of creatine synthesis or muscle-cell growth. It must reside in the central nervous system, and suggests that a change in attitude or capacity to push oneself to do things

perception

unsolved problems of heightened perception, to me at least, is how much is due to a stimulation of peripheral nervous systems, changes in

the ego and the id that was doing good. But she also became a little psychotic, and so her dosage of ACTH had to be lowered, and with every 10 units of ACTH one sonata disappeared. It all ended up with the same old music poorly performed.

Separating the ego from the id might involve separation of the cortical control from the lower vegetative system. We have had five patients with collagen disease who became psychotic, and whose

the head—and after this crisis I was surprised to see that she was perfectly cured of her polyarthritis. What is your opinion of thyroid function in such cases?

FOX: Did she receive large doses of cortisone?

MACH: Yes, and for ten days she was in a very severe state of delirium and psychic crisis.

present it doesn't seem to me that we can answer a question like that.

LEWIS. I think one must distinguish fairly sharply between the gross psychotic reactions, particularly the delirious ones, and these minor manifestations that Dr. Fox is drawing our attention to. We made a controlled study of highly vulnerable people, using placebos as usual in such a trial, and we detected no psychological changes that couldn't be accounted for either as a response to improvement in painful physical symptoms or as a result of predisposition evident in the patient's personality and evoked by many other circumstances besides cortisone.

Psychoses, I think, here fall into a different category from frankly psychogenic reactions. When they occur after cortisone or ACTH there is no question of expectation or suggestion, such as might arise in the ward your patients are in. quite a mythology often grows up in the

patients' minds where cortisone is concerned. But nothing of that kind can account for the gross psychoses that sometimes occur. As far as I know there is only one observation in the literature that seemed to throw light on the chemical basis of the psychotic reaction: that was the complete cessation of the psychosis after supplying potassium to a patient receiving ACTH who had severe potassium depletion at the time the psychosis developed. It is an isolated observation of Ransohoff's, and I doubt whether anyone else has repeated the finding.

It is commonly asserted that one shouldn't undertake cortisone therapy in people who have had previous mental illness or shown severe signs of predisposition to psychosis. I think this caution is unwarranted. The people we dealt with were particularly selected as having had a mental illness, and therefore were clearly predisposed, yet they didn't develop any mental disorder while receiving cortisone or ACTH. I think one must look for physiological and chemical phenomena rather than genetic or perhaps psychological ones, if one is going to account adequately for the occasional mental disturbance.

There is another point I'd like to ask Dr Fox about. He stressed perceptual changes, and particularly heightened awareness of reality. Now the main purpose I take it of his work so far has been to develop some hypotheses which can be formulated in a way that permits them to be tested. And I wondered if he would say what his hypotheses are regarding this perceptual change, which must be very different from what one sees with mescaline. I think we'll find out more about that with the help of further psychological tests on these patients. For one thing I still don't think we know what to look for in the personality before the substances are given. There certainly hasn't been a direct relationship, I agree, between a pre-existing psychosis and whether a person is likely to respond with psychosis to these substances. But as you study very carefully the patients who have reacted in this way, you do come upon much information concerning their background which may have a good deal to do with it. Particularly where psychosis is concerned, one very often finds a very "normal" person. It reminds me very much of the kind of thing we used to see in the Army, where the most acute psychoses seemed to occur in people who were regarded by those around them as particularly invulnerable to what was going on; it was this "brittle" personality, not flexible, who was able to stand stress up to a certain point but then when he broke he really broke down. We are trying to make our observations on these patients on the basis of projection tests, Rorschach and T.A.T., along with a very careful biographical study, trying to find out what kind of human relationships they have established with others, the extent to which the relationships really are rather brittle, and the patient's potential vulnerability even though there has been no actual breakdown.

ELMADJIAN: After ten years of working in this field at the Worcester Foundation, the thing that strikes me most is measurement. We've got to be able to measure something. We've then got to be able to say that what we measure is the same thing each time we do it. We've got to be able to say not only what we are measuring but how much of it is being

difficulties.

PHYSIOLOGICAL RESPONSE OF THE ADRENAI TO PSYCHOLOGICAL INFLUENCES AS INDICATEI BY CHANGES IN THE 17-HYDROXYCORTICOSTEROID EXCRETION PATTERN

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EVER since Walter B. Cannon (1929) described the rôle of adrenaline in mobilizing the organism for fight or flight, investigators of the human response to stress have recognized the special importance of substances elaborated by the adrenal gland. Selye's (1952) observations and hypotheses concerning the "alarm reaction" and particularly his explanation of certain psychosomatic diseases as an outcome of a "general adaptation syndrome" have emphasized the function of the adrenocortical substances.

Earlier studies relied on the fall in the eosinophil count as the most easily available index of adrenocortical activity. More recently the urinary excretion products of the carbohydrate-active steroids have been considered a more reliable index. Our concurrent biochemical and psychological observations of the pituitary-adrenal response to stress have been based on a method for the comparatively rapid measurement of urinary glucocorticoids as developed by Reddy, Jenkins and Thorn (1952). Unlike previous methods which measure only the free urinary glucocorticoids, this method detects the glucocorticoid metabolites both in the free and the conjugated form. The 17-ketosteroids were determined by a method which combines the ethylene dichloride procedure of Drekter, *et al.* (1952) with the colorimetric procedure of Holtorff and Koch (1940).

The psychological methods and interpretations were an outgrowth of certain psychoanalytic assumptions concerning the nature of stress. Mentally integrated activity is interpreted as an expression of biological drives and their derivatives. These drives preserve the life of the organism and press for their own discharge. Thirst, hunger, or the urge to breathe exemplify comparatively simple drives, also referred to as instincts. We assume that these instincts as well as the more complex sexual and aggressive urges respond to changes in the hormonal state of the organism. This results in quantitative biochemical alterations, shifts in the pattern of nervous response and qualitative transformations of perceptions and feelings.

During childhood the individual remains a relatively helpless prey not only to environmental perils but also to the annihilating strength of his own instinctual drives and needs. The conscience exemplifies the development of certain inner psychological controls which make it possible for the individual to find instinctual satisfaction in accordance with external reality. *Under certain circumstances the demands of* conscience may themselves become a source of stress. This morning, stress was defined as "any external threat, any intensification of instinctual drives or any increased demand from the conscience which challenges the capacity of the individual to maintain a balance between his needs and his satisfactions."

Selye (1952) has recently re-stated his interpretation of the adrenal-stress relationship He has emphasized that stress sensitizes the organism to respond to normal or near-normal amounts of adrenal hormone He describes certain manifestations of stress as resulting from hypercorticoidism, whether because of an actual increase in corticoid production or from sensitization of certain target organs to these hormones. Engel (1953), on the other hand, favours the view that adrenal hormone maintains homeostasis by facilitating a variety of metabolic and other reactions which are primarily determined by the needs of the tissues. He adds that "in all

likelihood the truth lies somewhere in between these two views with the adrenal and stress responses so inextricably interwoven and interdependent as to be essentially inseparable."

Our own successive observations of the adrenocortical responses of certain patients over relatively prolonged periods of time suggest that variations of adrenal activity constitute a sensitive indicator of psychological as well as homeostatic balances, quite aside from the physiological effects of the adrenal hormones themselves on the functioning of the organism.

In one such study (Rizzo, *et al.*, 1954) concurrent observations of behaviour changes and of adrenocortical variations were made in a cyclothymic patient during a period of over a year. Interpretation of these psychological and biochemical relationships must be cautious and tentative. The available biochemical methods for studying adrenocortical activity have been developed so recently that there has not been time to establish a "normal" or "usual" background with which to compare the results found in any given individual. Longitudinal studies help to reduce the confusing effect of so many unknown variables because the patient to some extent becomes his own control.

It was found that glucocorticoid excretion was abnormally low during the months of manic overactivity but rose to normal levels along with the patient's clinical recovery. During the period of overactivity, glucocorticoid values rose to normal levels in response to the administration of ACTH or cortisone on three occasions and twice in association with psychological situations which apparently constituted a challenge to the patient's specific emotional vulnerability.

Throughout the entire period of study the urinary 17-ketosteroid excretion remained within normal limits. This was so even on those occasions when ACTH or cortisone was administered. There was no apparent correlation between the blood eosinophil levels and the clinical state or the urinary steroid levels. Uropepsin levels were slightly above the normal

range during the period of overactivity and dropped abruptly to levels well within the normal range at the time of the patient's recovery. Laidlaw and Thorn have discussed the physiological implications of these findings and have suggested the interpretation that during the overactive period the patient's adrenal cortex was abnormal in the sense that there was a diminished synthesis of glucocorticoids in association with a normal synthesis of 17-ketosteroids. The output of ACTH from the anterior pituitary was diminished but the synthetic process for 17-ketosteroids was relatively uninfluenced by the decrease in pituitary output of ACTH. In summary, Laidlaw and Thorn considered it possible that this patient's adrenal cortex had always been abnormal, in the sense that whenever for any reason the anterior pituitary secretion of ACTH diminished, the secretion of glucocorticoids fell while that of the 17-ketosteroids continued in a relatively independent fashion at a normal level, thus resulting in a disturbance in the urinary 17-ketosteroid-glucocorticoid ratio.

The patient is a fifty-year-old woman who, since the age of twenty-one, has had periods of depression lasting for several months, alternating with phases of overactivity. During the past ten years the intervals of relative emotional stability have lessened and the severity as well as the duration of the mood disturbances have increased.

She has received psychotherapy for the past four and a half years. Her initial overactivity lasted for eighteen months and was then succeeded by depression for over a year, during which, at the age of forty-eight, she was admitted to a psychiatric hospital for the first time in her life. After leaving the hospital, several months of unstable transition were followed by another year of overactivity with excitement of such severity that she had to be re-admitted. Studies of her adrenocortical function began at the time of her second hospitalization. Observations were made throughout this period of excitement and have been continued since her discharge from the hospital and the resumption of her usual responsibilities—a period of over a year in all.

For several months during the period of greatest overactivity her glucocorticoids fell below 1 mg. per twenty-four hours—an abnormally low value. Three times during this "low" period the glucocorticoid level rose to normal in response to the administration of ACTH or hydrocortisone although these substances produced no observable effect on her mood or behaviour. The glucocorticoids also rose in association with two psychological situations that were especially threatening to her self-control. They increased during the twenty-four hours before she left the hospital for the first time in five months. She had begged to be allowed out, but this meant giving up a protection against the possibility of carrying out aggressive impulses toward her family or her friends. The glucocorticoids again rose after a stormy visit home during which she felt rejected by her husband and turned from him to an alcoholic woman friend—a situation which may well have been experienced as a repetition of her childhood disappointment in her father and a frustrated infantile appeal to her mother who also drank large amounts of alcohol. Other visits were accompanied by a certain amount of emotional tension, but the special circumstances of these two occasions aroused her anxieties to a much greater extent. In association with a gradual clinical recovery the glucocorticoid excretion returned to a normal level.

This patient was treated and observed by a psychiatrist and, although she had not been psychoanalysed, the clinical material has been psychoanalytically interpreted. The infantile regressive (oral) implications of manic depressive psychoses have been repeatedly demonstrated since Karl Abraham (1927) described them so vividly twenty-five years ago. This regression activates a ruthless drive for pleasurable intake, partially gratified during the manic phase (e.g., by phantasies of swallowing the loved object—in the words of this patient, "absorbing his essence") and expiated to some extent by punishment during the depressive self-attack. During "normal" intervals the cyclothymic personality proves vulnerable to any interruption of ego-bolstering influences

(Bibring, 1953). The loss of such support, more often in a setting of feeling jilted rather than merely saddened, brings the threat of raging self-destructive frustration. Manic overactivity attempts to deny the emotional loss and to externalize the self-destructive rage. The patient retrospectively described what she had felt during her most excited period—"not only is my philosophy to be happy no matter what the circumstances, but to exude happiness. My acting as a rule is so successful that I can nearly inwardly achieve the state. A psychiatrist can observe this and, therefore, realize that I am striving to overcome an unwelcome guest in my house." Thus a state which may be interpreted as one of psychological regression to a comparatively primitive manic denial of depression was found to be associated with a diminished excretion of glucocorticoids and a normal excretion of 17-ketosteroids.

The increase in urinary 17-ketosteroid-glucocorticoid ratio during a period of overactivity reflects a relative predominance of the anabolic over the catabolic phase of protein metabolism insofar as adrenocortical secretion is concerned. Could this represent the physiological counterpart of the patient's psychological defence against depressive drives which self-destructively threaten her own tissues?

Further observations on this patient, especially if she should again become depressed, and on others, will clarify the meaning of these early observations. Whether the adrenocortical pattern of response reported here will be found in other cyclothymic patients remains to be demonstrated.

M. Reiss (1953) at Bristol has investigated the day-to-day variations in the steroid hormonal excretion of mental patients. Since he used a different technique for measuring the glucocorticoids, we cannot compare our results with his. He has, however, reported that the excretion rates of 17-ketosteroids and of the glucocorticoids were not necessarily parallel and that these relationships shifted when the patient went from one emotional phase to another. Unpublished recent findings of a sharp rise in the glucocorticoid level of one of

our patients with acute excitement suggest that the bi chemical response is quite different in states of panic befor the defences have become relatively stabilized.

In the study just reported, an alteration in adrenocortic function was observed with the activation of certain infanti emotional conflicts. If future observations confirm this, may lead to the further elucidation of similar psychosomat problems (Margolin, 1953) and also of such closely relate psychosomatic disturbances as anorexia nervosa, obesity addiction and ulcer. We think of them as closely relate because clinical observation indicates that oral intake for a of the patients with these psychosomatic disturbances ha unconscious emotional implications in certain ways similar t the primitive phantasies expressed by some psychotics. Th fact that psychosomatic diseases such as ulcerative colitis c asthma sometimes alternate with severe depressions seem to confirm the hypothesis that there is a similarity in som aspects of the underlying psychological structure of patient having either a psychosomatic disease or a functional psycho sis. This is also suggested by the same special vulnerabilit to rupture of human relationships demonstrable during remissions from psychotic episodes or during intervals be tween bouts of severe psychosomatic disturbance.

It now looks as though the early attempts to assign specifi psychological conflicts to each of the so-called psychosomati diseases was somewhat misleading and at the present tim there has been a tendency to emphasize the basic similaritie *rather than the differences in the psychological* structure o patients suffering from any one of these diseases. We no longe think of the psychological conflict as the cause of the somati disturbance but are more inclined to think of both as expres sions of an underlying defective integration of the organism. We assume *that this defect in function can be observed in a* corresponding disturbance of neurological, hormonal, and psychological balances in response to stress.

Different people react to external events in various ways and we all know that the same person reacts differently at

various times. To study the effect of life stress, therefore, requires an intimate knowledge of the person. A psychoanalysis makes it possible to acquire this knowledge within the setting of a relationship to the therapist which reaches sufficient emotional intensity and has the advantage of daily (five times a week) interviews (fifty minutes to an hour each) continued for a period of two to three years. The technique of free association provides access to unconscious mental influences which become more clearly elucidated as the analysis proceeds. During each hour the patient reports psychological experiences which at first seem unrelated and diverse, but then converge toward a comparatively small number of underlying trends which the patient gradually learns to recognise with occasional help from the analyst who calls attention to apparent blind spots. This process gradually brings greater conscious recognition of unconscious drives and defences. We assume that the resulting change in the function of the organism is reflected neurologically by some sort of [illegible] stability of hormonal rhythms and balances.

Scientific observation in chemistry or physics includes consideration of the effect of the procedure itself on the data observed. This principle holds for psychology, of course, as well as for any other discipline. Psychoanalytic observation permits us to gain an understanding of the unconscious as well as the conscious effect of our procedures on the patient including the meaning of his relationship to the analytic observer (the transference).

A year ago we began the psychoanalysis of a thirty-four-year-old man with a duodenal ulcer Some months previously tarry stools had been followed by a severe hæmorrhage requiring a number of transfusions. He felt that his ulcer must be related to certain problems which included his feeling "blocked" from making any progress on a thesis for his Ph D. degree. He expressed an eagerness to get started in analysis,

hoping that it would make him more productive. He felt some anxiety, however, about whether the analysis would disturb him to such an extent as to make it more difficult for him to undertake a new course he was planning to teach in the coming academic year.

Before the beginning of the analysis he was asked to bring in a series of twenty-four-hour collections of his urine, and twenty-four-hour collections have been continued since. The 7 a.m. to 1 p.m., 1 p.m. to 7 p.m., 7 p.m. to 1 a.m., and 1 a.m. to 7 a.m. collections were examined separately. Volume, creatinine, 17-hydroxycorticoid and uropepsin determinations were made on each specimen. Laidlaw and Thorn were particularly interested in the diurnal excretion pattern of the 17-hydroxycorticoids which was found, before beginning the analysis, to correspond to most of the observations which had been made on healthy subjects. Much more of the steroid was excreted during the daytime than at night and the least amount was excreted in the 7 p.m. to 1 a.m. period. There was a slightly increased excretion between 1 a.m. and 7 a.m. when the patient was asleep. During the twenty-four hours including his first psychoanalytic interview the pattern was rather dramatically reversed with a much greater output of steroids during the night. This reversal recurred on a number of occasions during the early months of his analysis but this happened less frequently thereafter, even though his steroid excretion during the daytime sometimes reached levels found in patients with Cushing's syndrome.

The patient has not felt emotionally neutral either to the urine collections or to the psychoanalysis itself. In an early interview, e.g., he remarked that he felt he was much too excited about trying to do exactly what had been requested for the laboratory. He felt he had to do it exactly and right on the dot "otherwise Papa won't be pleased". This reminded him of his relationship to his own father. It was not so much direct orders from his father that he found difficult to deal with—he could usually oppose these when he wanted to. It was the "implied order" that he could not avoid carrying out.

He would try to hear what his father really meant no matter what his father actually said. He felt the same way about the doctor or whoever else was asking him to carry out these laboratory procedures. He added the next day that he had noticed the cute secretary at the laboratory, wondered whether she was the one who examines his urine, and what she thought about him as a man.

His emotional investment in the collections was manifested at other times by somehow "forgetting" to bring them in at all but expressing regret because he realized that since he had been under unusual tension we would probably have been particularly interested in specimens obtained during that period. During his 32nd interview he remarked, "I have the feeling that if I urinate in enough bottles and am a good boy and come on time that you will give me the book chapter by chapter." He added that he felt that if he told the analyst everything about himself, especially the nasty things, that the analyst "would take his soul, put it through a washing machine and give it back to him at the end of the two years all clean and dry." Later in the analysis his passive orality became more insistent and he complained that he brought his fee, his urine, and his blood but that all the analyst did was to sit silently sucking out his secrets.

Since we are still in the midst of making the observations on this patient, we are not yet in a position to arrive at definitive conclusions concerning our data. The material has been introduced for discussion because it illustrates a method for concurrent biochemical and psychological study of the response of the human adrenal to life stress.

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DISCUSSION

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his interviews summarized and charted.

had not encountered before in our studies was the almost complete disappearance of 17-hydroxy material, while the ketosteroids were maintained, not at minimum normal values, but at levels of 12–14 mg./day. We know that in acute mania, or in the acute anxiety state at least, that the ketosteroids may be very much increased. But this patient, as Dr. Fox explained, had been in this phase for some period when our particular study began. At first we thought that probably

values as being within one of our normal ranges. However, she has fallen quite close to one of the cases that Dr Wilkins described in which any tendency towards loss of normal menstrual periods and normal function might actually have been factors of secondary aggravation of her disturbance. It was curious that at the time when she was

red this

Did you

THORN: No, we have not done that. I would assume they might very well be there, and that this shift is in one area perhaps.

WILKINS: Dr. Thorn asked me about the interpretation of some of these results. I would admit that it is very important to take into consideration the relative ratios of the different types of adrenal functions (ketosteroids presumably representing androgenic functions) and comparing them with the other types of hormone function. However, I think great difficulty arises in the interpretation of urinary steroid excretion results, no matter what method of measurement is

determination to two different types of substances which are end-metabolites and are metabolized in different ways. I certainly would agree with Dr Thorn that there are some women with borderline cases of the adrenogenital syndrome in which the ketosteroids may not be excessively elevated, and in which there may be hirsutism and suppression of the menstrual cycles, anovulatory cycles, and that some of these women are helped by the administration of cortisone, but when it comes to asking me to interpret qualitative differences in adrenal responses from data relating to the types of urinary steroids such as presented, I must be excused

From a more general point of view, in a study of psychiatric material I feel that it is exceedingly important to have a very careful control about the dietary status and nutritional status of the patient, which often becomes very much affected and may shift markedly in psychiatric

function.

BROWNE. In connection with the day/night reversals, with regard to the ketosteroid rises we found those not infrequently in our acutely *ill* people or, when they were recovering, after a sleepless or disturbed night. I wondered whether, when these reversals occurred in the course of the analysis, there was unusual dream activity or the person was agitated and stayed awake all night

represented ...

us very much.

BROWNE: You say the problems don't come out. Can you date at what point in day or night those problems occurred?

FOX: Sometimes.

BROWNE: And apart from the dream content, what about sleeplessness, disturbance, thinking over the interview of that particular day—were those correlated?

FOX: To some extent. After a disturbing interview he would sometimes have stomach symptoms. On one occasion in an analytic session he was so disturbed that he got up from the couch and took some

of many days when the total excretion of material that we were measuring would fall well up in the Cushing's range.

LUFT: I should like to ask Dr. Fox, on the basis of his experience, if we can ever expect to find a regularly appearing diurnal rhythm of 17-ketosteroid excretion, and if so, can we then assume that this is a

before. Dr. Thorn's group has measured diurnal variation on some individuals, and we have some idea of how some people react, but it

in 1947 Phillips and I showed differences in the lymphocyte diurnal

normals and psychotics I think there is considerable background information.

At the Worcester Foundation we are now studying diurnal rhythms

[illegible] were two major correlations: the first was between the glucocorticoid excretion and the number of psychiatric interviews, and the second between the number of psychiatric interviews and the overactivity. The glucocorticoids were up during the period she was at home and were low when she was in hospital. I'd like to know whether Dr Fox has any views on cause and effect there, in view of the fact that Dr. Elton Mayo

*PINCUS, G., ROMANOFF, L. P. and CARLO, J (1954) *J. Geront.* 9, 113

long ago showed that interest in an individual increased his work output and efficiency in industry.

FOX: The patient was no longer psychotic when she was sent home. The difference between normality and overactivity was very well marked—she was so overactive that psychiatric hospitalization was necessary during that first period. The more subtle changes between overactivity and normality would, on the other hand, apply to the psychoanalytic patient, who was at work each day, so that the changes there might very well have some relationship to what goes on after he has had treatment. There we do try to eliminate that variable by seeing him every day.

THORN: The point you make, Dr. Cope, is that in her original situation, when she was quite ill, she was therefore seen at more frequent intervals. The reduction in interviews was occasioned of course by the fact that she improved.

s better?

It seems

to correspond closely.

FOX: Although it looks as though there was a tendency for it to become lower as she improved, the gastro-intestinal experts felt that according to the method they were using they couldn't really be sure that there was enough change to draw any conclusions.

BROWNE: Did she have any rise in uropepsin with ACTH?

THORN: She had ACTH for one day only, and I think Dr. Gray's feeling is that it may take several days for ACTH to have an effect.

ADRENOCORTICAL FUNCTION OF COMBAT INFANTRYMEN IN KOREA

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IN late August 1952 a team, consisting of personnel from the Army, Navy and Operations Research Office, went to the Far East to study the physiological, psychological and psychiatric aspects of combat stress of infantrymen. A preliminary report has already been made*. This is a presentation of the physiological and biochemical data compiled and analysed since that preliminary report, with emphasis on the adrenal cortical function and steroid metabolism. The data include urinary 17-ketosteroids, Porter-Silber chromogens, Na/K ratio, urea, uric acid and a few analyses of six individual 17-ketosteroids contained in the alpha fraction

Subjects

Two principal groups of men in combat were studied: (1) A group of men designated as "Able Co" which experienced an acute combat situation in which they were the leading company in an unsuccessful attempt to regain a hill position. Data to be presented from this group include a pre-combat (A) sample obtained some two hours after they were briefed for the attack; another sample (B) some seventeen hours after they were returned to the rear (this group includes only five men who were in the original group), and a four-day (C) and twenty-two day (D) follow-up after the principal engagement. (2) The second and smaller group consisted of men who experienced a prolonged and sustained action defending the hill position, after it was taken, against enemy counter-attacks for five days. No pre-combat (A) data were obtained

*Preliminary Report Technical Memorandum ORO–T–41 (FEC).

from this group which was designated as "George Co." However, data will be presented on (B) some fifteen hours after they were relieved and some ten days (C) after the defensive action.

These data are compared with a group of controls—men who were stationed immediately behind the main line of resistance in blocking position. Data of a group of psychiatric casualties will also be be presented.

A second aspect of the data to be reported includes a small sample of each group who were selected at random and to whom ACTH was given to test adrenal cortical reserve or responsiveness. Previous to testing the men in the combat area, a group of men in Japan (Camp Omiya) were given the ACTH in a test-retest situation to determine reproducibility in the same individual of the adrenal reserve of capacity.

Methods

Samples (not including the ACTH test) represent collections over a mean time of about three hours. Except for (B) samples of both Able and George Co. the majority of samples represented before-noon urines. Those receiving ACTH were injected with 2 ml. of Wilson's Gel preparation in the afternoon and collections made over approximately a fifteen-hour period; the last voiding being the following morning about 5–6 a.m.

The method for extraction of 17-ketosteroids was that described by Pincus (1945). The urine was hydrolysed with hydrochloric acid, extracted with ether, and the total neutral extract taken to dryness in Korea. The extracts in test-tubes were flown to the United States and steroid analyses were completed at the *Worcester Foundation Laboratories.* The butanol extraction for Porter-Silber chomogens and preparation of the samples for analysis were completed in Korea and sent in test-tubes to Dr. Peter Forsham at the Metabolic Institute at the Universtity of California Hospital, for the conduction of the Porter-Silber reaction. The method used was a modification of the Reddy Method (Reddy, Jenkins and

Thorn, 1952). Paper chromatography of alpha ketosteroids was conducted by the procedure described by Rubin, *et al.* 1953.

Results

Control and Combat Data

In Fig. 1, we present the mean 17-ketosteroid output values as mg. per hour in the various control and combat groups. Able Co. A minus officers has been set up separately for two reasons: (1) officers contributed urines to the pre-combat (A) series but not to the subsequent ones, and (2) the officers exhibited, as a group, an extremely high output

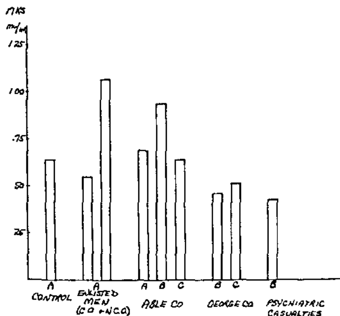


FIG. 1. Urinary 17-ketosteroid excretion. From left to right the first (A) represents the mean value of the controls, the first column under the second (A) represents the mean of the enlisted men in Able

level of 17-ketosteroids (17-KS). The average (A) output value for the five officers was $1 \cdot 07 \pm 0 \cdot 187$ mg. per hour, whereas the corresponding value for the fifteen enlisted men was $0 \cdot 56 \pm 0 \cdot 6$ mg. per hour. It is deduced that either these officers at any rate were a group of "high" 17-KS excretors (Pincus, *et al.*, unpublished), or that emotional tension attributable to battle anticipation led to especial activation of the pituitary-adrenal system during the pre-combat period.

When mg. per hour pre-combat values (A) are compared with post-combat values (B) for Able Co., a not quite significantly higher value is obtained for the latter period. In contrast, the post-combat values (B) for George Co. are significantly lower than both the Korean control and the post-combat (B) Able Co. values. There are no other statistically significant differences between the various sets of data. It is notable, however, that the psychiatric battle casualties exhibit the lowest mg. per hour output, indicating no stimulation, but rather a damping of the 17-KS output.

The Na/K ratios are depicted in Fig. 2. We note that in the control group we have fairly good agreement between A and A^1 (test-retest at one week interval), somewhere around three as a value. However, in observing the data on Able Co. we find that A of Able has a relatively low ratio, indicating some degree of adrenal cortical activity with respect to electrolytes and, furthermore, that it is still low in B. However, in C value we find that the value comes back towards normal, in fact a little above normal, and that this continues in D. With regard to George Co. we find that the ratio is high on the average, indicating hypoadrenal activity. Then some nine to ten days later we find that the value has returned back towards normal. The psychiatric group shows a low Na/K ratio, indicating some degree of adrenal cortical activity, in fact the same degree of activity as that of Able A or B, but with a very low 17-KS.

Fig. 3 shows the urea nitrogen in terms of mg. per hour. We note that in the Korean controls A and A^1 we have fair agreement, namely, within 100 to 500 mg. urea nitrogen

per hour. In A of Able there is an increase; this increase is only of marginal significance. In B we have a real increase in the urea nitrogen, indicating a significant protein catabolism (almost doubling the urea nitrogen output above that of the normal controls). In C we see this return of urea nitrogen back towards its normal value and in D a rather slight increase, but not significant. In the case of the chronic stress

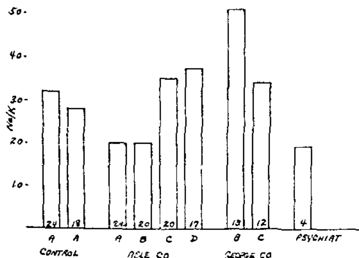


FIG. 2. Urinary sodium/potassium (molar) ratio Numbers at the base of the open columns indicate the number of determinations included in each group.

situation we find that rather than a nitrogen catabolism, the value is in the normal range, and in the post-stress the C value increases somewhat though not significantly. The psychiatric group shows a decreased urea output This value is quite low and significantly lower than the controls. It should be pointed out that in the B of Able the increased urea nitrogen excretion is reflected in the blood with a very significant high urea value; almost 20 mg. per cent urea nitrogen in the blood

In Fig. 4 we have a scatter diagram of the Na/K ratio against the 17-KS of Able Co. B, indicated by the dots with the circles around them and the X, indicating the George Co. B group. The correlation coefficient of the Able group was —0·6 but was not significant. However, we note that the extreme values in Able Co., those who had a low 17-KS,

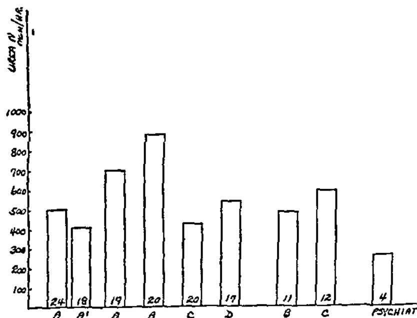


FIG. 3. Urine urea nitrogen excretion. Figures at base of open columns indicate number of determinations included in each group.

are those who have a high Na/K ratio and the converse, the low Na/K ratio individuals, are those who have a high 17-KS. We note that in the George Co. (the X's) they are scattered along the low 17-KS and high Na/K ratio area.

Figure 5 shows the urea nitrogen in mg. per hour against 17-KS in mg. per hour. We have a very good correlation of +0·74 and a significance of <0·01. Here again we note that the data of George Co. B are clustered around the low 17-KS

and low urea areas. We obtain the same picture as that with the urea when uric acid is plotted against 17-KS. The r is +0·54, with a significance of <0·01.

In general, we see from these data that Able Co. B shows increased adrenal cortical activity in the indices examined,

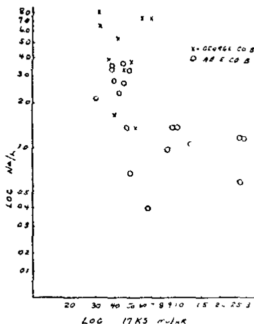


FIG. 4. Relationship of the Na/K ratio with 17-ketosteroid excretion of both Able Co. B and George Co B.

such as 17-KS and Na/K, with catabolism, as shown by the urea and uric acid output The catabolism is evident also with higher creatinine values. This is not apparent in the chronic group, namely, George B, where we have no increase in 17-KS and the Na/K is high; the nitrogen metabolism is in the normal range. In the psychiatric group we find that there is a low 17-KS, but, unlike George B, a low 17-KS with some degree of electrolyte activity.

ACTH Tests

In Table I are the 17-KS data on the group of seven soldiers stationed at a rehabilitation centre in Japan (Camp Omiya) and subjected to an initial (A) and a repeat (B) ACTH test with urine collected for the fifteen hours following ACTH administration. No pre-injection samples were determined.

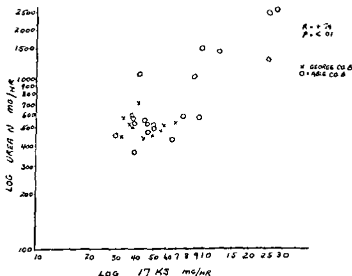


FIG. 5 Scatter diagram of urinary urea nitrogen excretion and the 17-ketosteroids of both Able Co. B and George Co. B.

Whether the data are calculated as mg. 17-KS per hour or per 100 mg. creatinine, remarkably high and significant correlations between the data of the two tests are obtained, indicating that with the ACTH used the 17-KS response is remarkably uniform and highly characteristic for each individual.

Figure 6 represents the mean data of 17-KS for various subjects in the ACTH test. It should be noted, first of all, that the Korean controls exhibited no increase of 17-KS

Table I

REPRODUCIBILITY OF ACTH TEST*

	17-KS, mg /hr		17-KS/ 100 mg Creat	
	A	B	A	B
M. 7856	0·52	0 57	0 69	0 70
R. 5249	0·21	0 16	0·38	0 67
W. 9926	0·62	0 51	0 90	—
J. 1789	0·73	0 73	1 14	1 11
M. 2878	0·24	0 35	0 55	0 51
M. 7056	0 23	0 29	0 30	0 40
P. 9139	0 06	0 15	0 10	0 26
	r=0 95		r=0 94	

*Time of each collection represented urine collected from 3 00 p m to 7 00 a m thus including overnight sample. ACTH given about 3 00 p m

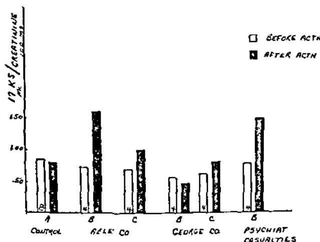


FIG. 6. 17-Ketosteroid excretion mg /100 mg. creatinine before and after ACTH. Number at the base of open columns indicates the number of individuals tested.

excretion following ACTH administration. Since the pre-injection urine collections were made between approximately 9.00 a.m. and 12.30 p.m., and the post-injection collection for the following fifteen hours, which included a period of sleep, this apparent lack of stimulation may in fact involve sufficient stimulation to restore toward the morning level the known decline in 17-KS output values occurring in the afternoon and during sleep (Pincus, 1943).

In any event, in contrast to the control subjects, the men of Able Co. tested show increases of 17-KS output following

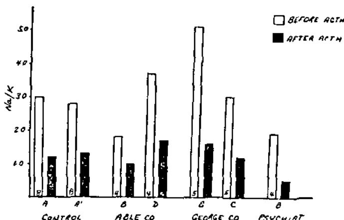


FIG. 7. Urinary sodium/potassium (molar) ratio before and after ACTH, number at the base of the open column represents the number of individuals included.

ACTH both in the post-combat (B) period and ten days later (C). The George Co. subjects, on the other hand, demonstrate a decline of 17-KS output following ACTH (which is particularly obvious in the mg. per hour data) during the B period, but ten days later during ACTH an output increase occurs. The psychiatric casualties demonstrate a significant increase of 17-KS per 100 mg. creatinine in the post-combat period.

Figure 7 contains the Na/K ratio before and after ACTH. We find that on A and A[1] in eight individuals we have fairly good agreement in adrenal sensitivity as noted by the Na/K

ratio. We find that in B of Able the adrenal responds to the ACTH even though the pre-samples already indicate some degree of adrenal activity. In D sample (C samples were lost) we find that the Na/K ratio is high, that is back to normal in the pre-ACTH samples, and is responsive to the ACTH by a reduction in the Na/K ratio. Now the interesting feature is that of George Co., where there is a hypoadrenal-cortical activity as indicated by the Na/K ratio. There is, however, a response of the electrolytes after ACTH, though there was

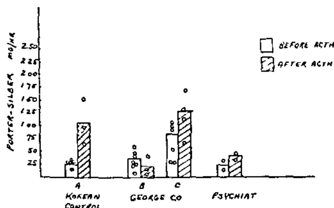


FIG. 8. Porter-Silber chromogens before and after ACTH (see text).

no such evidence in the 17-KS. (We will note next that the 17-KS are about the same as the P-S). The values in C return back to normal. In B of the psychiatric group we find that though the adrenals are active there is much more activity in terms of electrolytes after ACTH.

In Fig. 8 we have the Porter-Silber (P-S) chromogens in terms of mg. per hour in three separate groups: (1) the Korean control, (2) George Co. B and C, and (3) the psychiatric group. Our Porter-Silber data were scattered because only a portion of the total samples extracted were analysable. This in all probability is due to the high blank and other defects in this particular method used, as well as possible technical errors

in preparation for analysis. In our Korean controls there are only a few determinations. The first block indicates the pre-ACTH group and is followed by the post-ACTH. In these particular data the post-ACTH group do not include any of the individuals in the pre-ACTH group. There is a clear increase as a result of ACTH injection. In B of George Co. we notice that there are some seven determinations for pre-ACTH and three for post-ACTH. The three individuals in the post-ACTH data are included in the pre-ACTH group. We note that the adrenal is non-responsive as measured by the P-S reaction. However, in C we note that there is a response to the ACTH after nine to ten days, and the four individual determinations are included in the pre-ACTH. In the psychiatric group the two pre-ACTH are not the same individuals as the post-ACTH. We note here that two features stand out: (1) there is in control samples a greater increase in the post-ACTH in the P-S than there was in the 17-KS, and (2) that the adrenal non-reactivity observed in B of George Co. in the 17-KS is confirmed with the P-S reaction. However, in the psychiatric group there is a difference—we have an increased reactivity of 17-KS after ACTH, but we do not have such a phenomenon in the P-S reaction. The data in this group are admittedly small in number and rather scattered, but we feel that these data are at least internally consistent and indicate a differential steroid excretion.

In Fig. 9 we have the 17-KS determinations on which we also have P-S. The scatter diagram represents P-S mg. per hour against 17-KS mg. per hour. We note two features in these data: (1) from about 0·3 mg. of the 17-KS per hour excretion to about 0·7 mg., we find the distribution of these points such that a small increase in 17-KS is related to a greater increase in the P-S. However, we note that after this critical point of somewhere between 0 7 to 0·8 mg. per hour, the 17-KS continue to increase but that the P-S titre decreases. On inspecting the data we noted that the distribution between 0·3–0·7 mg./hr. 17-KS consisted mostly, if not all, of control values, while those where the 17-KS values are higher than

the 0·7–0·8 mg. per hour level are stress samples. We feel that from these data some fundamental aspect of steroid metabolism is revealing itself here, especially with respect to stress.

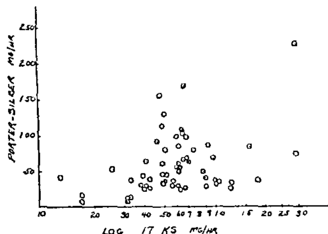


FIG. 9. The relation of the 17-ketosteroid and Porter-Silber chromogens determined on the same samples.

Discussion

In the comparison of an acute stress against that of a chronic stress situation, a large number of indices indicate that the biochemical status, or say biochemical profile, of a chronic battle situation is quite different from that of the acute. Whereas in the acute state there is an indication of increased steroid output with increased protein catabolism, we find that in the chronic state (as far as the steroid measures are concerned) there is a dulling of the adrenal cortical function and no protein catabolism. However, it should be clear from the electrolyte data observed in the chronic stress situation after ACTH that the adrenal is not *completely* non-responsive; it is non-responsive as far as 17-KS and P-S are concerned, *but it does respond to ACTH where some hormone apparently related to the electrolyte function is stimulated.*

It may be inferred that whatever hormones are being stimulated after ACTH (acting on the electrolytes) in the chronic group, these hormones are not the compounds detectable in 17-KS titre or in the P-S titre. Since it has been demonstrated that ACTH does not increase the excretion of electrocortin, the suggestion may be made that the steroid responsible for this effect could be Compound B (corticosterone). The metabolic products of B would not appear as 17-KS and probably only slightly as P-S chromogens. In the pre-ACTH psychiatric group we see that the 17-KS and the P-S indicate a lower adrenal cortical function level, but with some degree of electrolyte action present. Furthermore, when ACTH is given in the psychiatric group, there is a marked 17-KS response, *but the P-S is not very great;* as a matter of fact it is clearly less than normal. The electrolyte response is very sharp. The lowest electrolyte ratios are observed in the psychiatric group. Reviewing the data then, each particular group, namely, the controls, the acute group, the chronic group and the psychiatric group, has a different "biochemical profile" and can be differentiated one from the other (Table II).

Table II

SUMMARY

Control Data	*Acute*	*Chronic*	*Psych*
Urine			
17-KS 0 64 mg /hr.	(+)	(−)	(−)
P-S Chrom. 0 51 mg./hr. .	(+)	(−)	(−)
Na/K (molar) 3 0	(−)	(+)	(−)
Urea 450 mg. urea N/hr.	(+)	n	(−)
Uric Acid 30 mg /hr. .	(+)	n	n...
ACTH			
17-KS (+) .	(++)	(−)	(++)
P-S. Chrom. (++) .	(−)?	(−)	(−)
Na/K (−) . . .	(−)	(−)	(−−)

Since the pre-ACTH psychiatric samples revealed low 17-KS, even though the individuals showed marked emotional

display and apparent stress, there is a suggestion of a disturbance of the pituitary-adrenal axis. After ACTH there was a marked adrenal response as indicated by the increased 17-KS output and the reduced Na/K ratio (though the P-S titre was low); therefore, the disturbance is more likely to be at the pituitary level. The low P-S with the high 17-KS may be explained as due to more rapid conversion of C_{21} to C_{19} steroids.

The discrepancy between the P-S and the 17-KS values, namely, (1) the higher P-S values after ACTH in the control samples, and (2) the finding that the stress samples showing high 17-KS have low P-S, may permit the consideration of the following hypothesis: The adrenal cortex in the first stages of stress produces C_{21} steroids in large amounts with some C_{19} components. As the stress condition continues either the adrenal ceases to produce increased amounts of C_{21}'s with an increment in favour of C_{19}'s, or the C_{21}'s are rapidly converted to C_{19}'s in their intermediary metabolism. If we tie in the fact that there was in the acute stress increased nitrogen excretion, while the nitrogen excretion in the chronic stress situation was low to normal, it is not difficult to observe the similarity of the above suggestions to the previous hypothesis of the "S" and "N" hormones of Albright (1942–3) and Browne (1945).

Further evidence to clarify these points of interest may be obtained by chromatographic analyses of the alpha ketosteroids, especially by ascertaining the quantity of oxygenated derivatives of hydrocortisone and cortisone.

only those of urine analyses relating to adrenal physiology.

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DISCUSSION

ELMADJIAN. Recently Enrico Forchielli of the Worcester Foundation has been able to do chromatographic analyses of the ketosteroids in two individuals. The samples consisted of 10–15 hour urine collections after a dose of 160 I.U. of Wilson's gel preparations. First I should like to present the general scheme of metabolism of adrenocortical steroids (Fig. 10). This shows three types of adrenal steroids—Compound F (C_{21}) and two types of C_{19} compounds, 11-deoxy and 11-oxy C_{19} compounds.

According to this scheme, the first step in metabolism is the reduction of Compound F to tetrahydro-E or tetrahydro-F (measured by the

ALPHA 17-KETOSTEROIDS IN URINE

FIG. 10.

Porter-Silber reaction) and these steroids are then further metabolized to 17-ketosteroids. According to Dorfman's work these are mainly the 11-oxy-ætiocholanolone (5β) type of compound, which during acid hydrolysis forms Δ^9-ætiocholanolone. The ratio between 5β and 5α compounds is 5.1.

[illegible]

sterone.

Fig. 11 shows the chromatographic pattern of two individuals. F-8576C is an individual ten days after battle (which lasted five days)

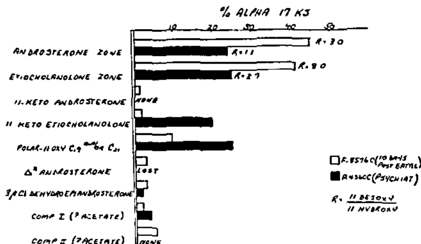


Fig. 11. Chromatographic pattern of 17-ketosteroids of two soldiers after battle.

whom we consider to have recovered to normal after a chronic stress, his data are consistent with our normal data The other subject; P-4360C, is a psychiatric casualty one day after the end of the battle. In the chromatographic technique the Δ^9-androsterone and the Δ^9-ætiocholanolone can be separated out from their parent compounds, *namely the androsterone and the ætiocholanolone, only after undergoing* oxidation to the epoxy compound. Therefore these are referred to as "zones", indicating that these are mixtures of two compounds After oxidation we can find out how much of the mixture is androsterone or Δ^9-androsterone, and how much of it is ætiocholanolone or Δ^9-ætiocholanolone. "R" indicates the ratio of the 11-deoxy type to the

11-hydroxy type. In F-8576C the androsterone zone "R" is three, which is the value obtained in our other normals. However, the ratio of the psychiatric sample is 1·1, indicating that there is relatively more 11-hydroxy material in this sample, and that this particular sample has about twice as much or three times as much of the derivative from the 11-oxy C_{19} type of compound as F-8576C. In the ætiocholanolone zone the ratio is about 8–12 in normal subjects. F-8576C has an "R" of 8·0. In the psychiatric subject the ratio has dropped to 2·7, indicating that there are more 17-ketosteroids derived from C_{21} compounds in the psychiatric sample than in the other. In the 11-keto-*androsterone, which is derived from the 11β-hydroxy Δ^4*-androstenedione or adrenosterone type of compound, there was not much change.

Now comes a very striking feature. A very small amount of 11-keto-ætiocholanolone (3 per cent) was found in the urine of F-8576C, but the psychiatric subject had a very high amount of 11-ketoætiocholanolone (18 per cent), which is derived from C_{21} compounds, or specifically Compound F. The next fraction is not completely differentiated yet; it is a polar fraction, containing an 11-oxy C_{19} and (or) a C_{21} compound. In this fraction we again note a large amount in the psychiatric sample. The next two compounds are present in the same amounts in both individuals and are in the normal range. These last two compounds have not been ascertained, we only know that they are acetates.

Table III

PRECURSORS OF TOTAL "ALPHA" 17-KETOSTEROID FRACTION*

	Classification of Precursors (Per cent contributed to the total alpha fraction)				
	C_{21} *Steroids*	C_{19} (11-oxy) *Steroids*	C_{19} *Steroids*	*17-Keto-steroid mg/hr*	*Porter-Silber mg/hr*
George Co.					
F-7856B	25·7	13·0	66·5	0·42	—
F-7856C	15·7	13·7	74·9	0·63	1·07
Psychiat					
P-4360B	61·0	11·5	20·0	0·78	—
P-4360C	48·6	11·2	35·7	1·24	0·34

*All collections made after ACTH (100 I U) was given Collection period ranged from ten to fifteen hours

Table III shows the [illegible] alpha 17-ketosteroid fraction of the [illegible] F-7856B represents urine collected immediately [illegible] battle. Sample F-7856C was collected ten days after the battle. P-4360B was collected during the battle period, and P-4360C from the same subject two days later. We note in F-7856B and F-7856C that only a small percentage of the 17-ketosteroids are derived from C_{21} compounds, and

the percentage derived from adrenosterone or 11β-hydroxy-Δ^4-androstenedione is about the same. Here we have a very high proportion of C_{19} derivatives. However, when we consider the psychiatric samples, P-4360B and P-4360C, there is a marked difference. In this subject the amount contributed by C_{19} compounds is only about half the value of the normal subject, but there is increased amount from C_{21}. The amount

derivatives.

The interpretation of 17-ketosteroids remains complex. I wish merely to point out that I think it is very important in the future to chromatograph the 17-ketosteroids. If we do that I think much of the confusion will disappear in this particular type of work.

initially?

ELMADJIAN: We can't tell We took a random sample, and the samples where we gave *ACTH* did not include any individual who had a high 17-ketosteroid; they all had low normal 17-ketosteroids.

co-operated very well, followed directions as if it were any other military matter. Not every sample was taken by the same person. The rest of us waited in the laboratory for the samples to come in.

THORN: It is important to point out that the detailed chemical fractionation of specimens collected under these reasonably good

measurements?

ELMADJIAN: I am only reporting as a member of the team, not as one who knows about psychological measurements. The tests requested by the Army were for higher mental functions. We had four psychologists who conducted these tests. Under the conditions of the tests the psychological determinations were not very promising and showed nothing. The psychiatric aspects of this study were very difficult, because the psychiatrist couldn't see each individual for long; he had about twenty minutes of interview each time he saw him. We have a mass of data along the following lines: the developmental history of each soldier, i.e. when his father and mother died, how many people in the family, whether he played competitive games or single games—football or golf, etc. I have all the raw data; no psychiatrist wants to touch it.

GENERAL DISCUSSION

GUILLEMIN: We have been interested in another link in the chain of events which results in pituitary-adrenal stimulation by stress, namely the

pituitary.

cological bl

had been obtained by using this technique, because pharmacological blocking drugs are endowed with very strong corticotrophic properties. In our experiments we have injected over

properties of these substances. We have thus achieved a state of dissociation between the non-specific corticotrophic effects and the

(as measured by concentration of the adrenal ascorbic acid). Pretreatment with dibenzyline (SKF-688-A), atropine and phenergan (3277-R.P.) respectively, prevented the ACTH discharge normally induced by noradrenaline, acetylcholine and histamine.

From these results I think we can conclude that none of these humoral agents can be taken as the "first mediator" which will directly initiate the release of ACTH by the anterior pituitary, nor can any of these substances be considered as the central mediator which would be liberated by some hypothalamic

link at any moment for the release of ACTH by the pars distalis under any conditions of stress.

FORSHAM: This seems to be a death knell for the direct stimulatory action of adrenaline and noradrenaline on the pituitary-adrenal system.

BROWNE. Is there not a difficulty in that regard, if one goes back to the concept of acetylcholine and the transmission of parasympathetic nerve impulse. You recall in the intestine in

Auerbach's plexus it was impossible to interfere with the nerve action by administration of atropine. That was regarded as a proof that the acetylcholine was not a mediator substance. I understand that it was found that it was perfectly possible for acetylcholine to be a mediator locally even at a time when inhibitors were present in the general circulation. However, in this case of Auerbach's plexus the acetylcholine is liberated in direct contact with the cells on which it acts, whereas in the case of the hypothalamus and pituitary there is presumably some distance between the hypothetical site of liberation and the site of action.

GUILLEMIN: In view of this criticism, in the particular case of acetylcholine, we were able to show that systemic pretreatment with atropine could prevent electrocorticographic changes produced by topical application of acetylcholine on the brain cortex. Thus a phenomenon centrally mediated by acetylcholine was prevented by the administration of atropine.

ELMADJIAN: May I make a comment about adrenaline, *noradrenaline and 17-ketosteroids in human urine? We have done* diurnal variations of adrenaline, noradrenaline and 17-ketosteroids on samples from normal and psychotic subjects (unpublished data). There was no quantitative relationship between the amount of adrenaline, noradrenaline or 17-ketosteroids found in the same sample. While you are asleep the adrenaline is very low; then in the first four to five hours of waking the amount of adrenaline in the urine is from 1–25 times the amount when you are asleep. Yet the 17-ketosteroids only increase by 60 per cent.

LUFT: Did the test subjects eat in the morning?

ELMADJIAN: I did measurement with and without food; it didn't make any difference.

LUFT: We did the same thing. But if we keep the students lying down on a couch and eating, we don't get that increase in the adrenaline excretion during the morning hours.

ELMADJIAN: What about the noradrenaline?

LUFT: Experiments regarding noradrenaline are at the moment being performed in collaboration with Professor von Euler.

ELMADJIAN: *Our subjects were active; exercise in this case might* have a great effect on the fact that adrenaline goes up. One of our subjects was a chemist who has arthritis, and at one time he had seven times as much on waking as in the overnight sample, and the next time he had 50 times as much. In the latter case he had an acute attack of arthritis.

THORN: I should like very much to hear Dr. Bayliss's findings on diurnal variation.

Bayliss: In view of what Dr. Thorn said earlier, any contribu-

steroid levels, but this is not altogether satisfactory because in some subjects emotional disturbances may influence the results and obscure the diurnal variation. Many people have relatively

the plasma steroid level. For example, a neurologist colleague had levels constantly at about 9 μg. per 100 ml. for several successive days and then showed a concentration of 22 μg., this being on a day when he was going to Edinburgh to give an important lecture. We have followed the steroid levels in other colleagues who are

urinary steroid excretion.

When we came to study two R.A.F. volunteers who had been on bed-rest and a constant two-hourly intake of milk, butter and biscuits for three weeks, we found that both showed the expected diurnal variation in the urinary excretion of water and sodium. One showed a diurnal variation in plasma steroid concentration, the lowest levels occurring during the night and a high concentration at about 6 a.m., which I think other workers have observed. The other subject showed progressively higher plasma steroid concentrations in each four-hourly blood sample until the level reached 28 μg. per 100 ml. Now this second subject was apprehensive and did not like vene-punctures although he tried to hide this by becoming engrossed in a magazine when the samples were being collected. I think these high values are related to the emotional disturbance and we have observed the same effect in several other subjects suffering anxiety, tension or fear.

If the subject is at ease, diurnal variations can be detected both in the blood and in the urinary excretion. The renal clearance of 17-hydroxycorticosteroids is about 1 ml. or less per minute, which suggests perhaps that there is renal tubular reabsorption. Sometimes during two- or four-hourly collection periods higher levels are found in the blood in the period preceding that in which the urinary excretion increases—as though there was a "spill-over" into the urine. The diurnal variation in corticosteroid excretion

is not related to the diurnal variation in sodium and water elimination.

QUERIDO: I think that all the experiments that are to be reported in future must fulfill the requirements that Dr. Bayliss and Professor Borst and Professor Gerritzen have set up, with regard to keeping the subject in bed and giving constant water and caloric intake. We can't get anywhere in diurnal variation studies by putting the people under all kinds of stress or activity or metabolic changes, etc.

There is another point which I should like to bring out—that this rhythm is absolutely unexplained. You have to take into consideration permissive actions. For instance, I once had a patient with panhypopituitarism who had a complete absence of rhythm, absolutely constant excretion of water, sodium and potassium. He was treated with testosterone implantation, 600 mg., and after we had got him in better shape he showed a normal rhythm. I'm sure that it was not through testosterone itself, but just the permissive action of getting the patient into a better situation. The same holds for another case of a patient with panhypopituitarism treated with DCA, where also the rhythm came back.

THORN: We have followed the urinary 17-hydroxycorticosteroids of a number of individuals over many days, and have made a number of sub-divisions within the twenty-four hour period. After making quite an intensive study of the three-, four-, and six-hour fluctuations, we have decided for long-term studies to use twelve-hour periods, i.e. 7 a.m. to 7 p.m., and 7 p.m. to 7 a.m. We realize of course that in an individual case this may not be the most effective dividing point. We've studied some individuals over a period of one hundred to one hundred and fifty days, and only 10 per cent of the time will the night values for urinary 17-hydroxycorticosteroids be greater than the day; in other words, the ratio greater than 1. About the lowest ratio that one encounters in terms of the night versus day is 0·5. The 10 per cent reversal ratios in normals may vary considerably—one individual showed a shift 30 per cent of the time. This particular individual is an effective person in the lab who tends to push himself at night. It is possible these values may reflect his activity in this regard.

Patients with Cushing's syndrome whom we have investigated do not exhibit the day-to-night variation and their values stay up all the time. I imagine that in an early case of Cushing's syndrome there would still be some tendency for night to be less than daytime excretion. If one infuses hydrocortisone into an

Addisonian patient, after twenty-four hours one tends to get a fairly constant excretion, which again makes us feel, as has been mentioned here several times, that the renal factor is not the major factor. Changes in diurnal excretion seem to reflect a change in secretion rather than in metabolism.

[illegible]

more effective.

I am interested in Dr. Elmadjian's results. I wouldn't think you could go too far in drawing conclusions about psychotic versus normal individuals if you realize that normal people can exhibit marked shifts; although I am tempted to believe that those who have a more constant metabolic situation day and night obviously probably have less fluctuation.

We do know two things which are borne out by Dr. Bayliss's observations: that the reduction in secretory activity comes before one *quietens down in the evening,* and that the *initiation* of increased secretory level precedes awakening and getting out of bed. If we were to change the period of collection, to make a greater [illegible]

same way.

THORN: I think Professor Querido's point about electrolyte excretion is most important. In these studies renal function is an

, for such studies, follow a much

... Has anybody tried the lymphocytes? The lymphocytes go up from 2,000 absolute count to 3,500 or 4,500 at night. Of course one is making the assumption that this is measuring adrenocortical function. And I wish to warn that later we found that the administration of food, hyperglycæmia, would cause a decrease in blood lymphocytes. But if those things are controlled you might be able to study diurnal rhythm by that very simple method.

BROWNE: Does anyone know whether workers who *regularly* change from day to night work have any change in diurnal rhythm?

FORSHAM: Diurnal variations in night workers were found to be the same as those of day workers by F. Tyler and his associates.

THORN: That would be our assumption But it would be very interesting to investigate in any working group those people who adapt more successfully to night work.

BROWNE: Robert Louis Stevenson in "Virginibus Puerisque", characterized students as Student Owls and Student Larks—the

kfast.

, published

changed his

diurnal rhythm of 17-ketosteroids in the opposite direction by working at night and sleeping in the daytime.

BROWNE: Would one call Pincus an owl or a lark?

* * * * *

we seem to be

ment for Addison's disease, the treatment for Cushing's syndrome, treatment for adrenogenital syndrome, the possible areas of usefulness, or exploration at least, of adrenalectomy and hypophysectomy.

We still know very little about what is actually happening at the tissue level in response to a change in hormone concentration. There I believe Dr. Currie's remarks were very helpful, the difficulties with which he is presented in trying to interpret what is happening to a gland

by histological or histochemical methods are appreciable. We might say that this problem can be resolved in other areas, by chemical determinations, but I think that Dr Elmadjian's report today makes us all realize with greater humility than ever how careful one has to be in drawing conclusions from limited chemical observations. So in the area of chemistry as well as that of histology of the adrenal there is much to be learned.

As we move into the clinical field, and consider organs and finally the entire organism, we have greater difficulties, particularly in the nature of controlling experiments. The discussions today were particularly helpful with regard to the plan and attack of psychological and emotional problems. Professor Zuckerman and Professor Harris have this major difficulty in what would have seemed to us at the clinical level to be relatively simple anatomical questions.

On the other hand, as the observations from different disciplines accumulate they seem to reinforce one another. Dr. Elmadjian has shown the possibilities by which the adrenal may shift the nature of its hormone secretions, and there are spontaneous changes which have been observed which would support this idea The major problem is, how much are these characteristic shifts due to a pre-determined individual capacity in terms of metabolic pathways, secretory responses, and how much can they be generalized in terms of particular stresses (physical or emotional) For example, when one gives ACTH for a continued period of time, how wide will the variations in response of different individuals be? From the earlier studies of Dr Dobriner and some of the studies of the Worcester Foundation group we know that individuals vary considerably in terms of the substances produced by even a normal adrenal under normal circumstances.

Finally, I should like to state that this has been an extremely stimulating atmosphere, the capacity of the group to exchange ideas and to profit from each other's experiences has been outstanding. I am certain all of us feel as I do a great debt to the Ciba Foundation for being able to arrange a meeting of this type. In meetings of a small intimate character one has not only an improved opportunity for

well as

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duties

mportant element, and one must, for such studies, follow a much more rigid metabolic set-up.

ELMADJIAN: Has anybody tried the lymphocytes? The lymphocytes go up from 2,000 absolute count to 3,500 or 4,500 at night. Of course one is making the assumption that this is measuring adrenocortical function. And I wish to warn that later we found that the administration of food, hyperglycæmia, would cause a decrease in blood lymphocytes. But if those things are controlled you might be able to study diurnal rhythm by that very simple method.

BROWNE. Does anyone know whether workers who regularly change from day to night work have any change in diurnal rhythm?

FORSHAM: Diurnal variations in night workers were found to be the same as those of day workers by F. Tyler and his associates.

THORN: That would be our assumption. But it would be very interesting to investigate in any working group those people who adapt more successfully to night work.

BROWNE: Robert Louis Stevenson in "Virginibus Puerisque", characterized students as Student Owls and Student Larks—the former was bright at 2 a.m but was ineffective at breakfast.

ELMADJIAN: Dr Pincus did an experiment on himself, published in the first Laurentian Hormone Conference, where he changed his diurnal rhythm of 17-ketosteroids in the opposite direction by working at night and sleeping in the daytime.

BROWNE: Would one call Pincus an owl or a lark?

* * * * *

THORN: In the past three days we have heard of the results obtained in a large group of adrenalectomized patients, in hypophysectomized patients and in a large number of patients with Cushing's syndrome, and Addison's disease. It would appear that therapeutically, as far as the major disorders in adrenal dysfunction are concerned, we seem to be on very sound ground. Whether our basic knowledge is correct or not, we do not know, but it is possible to carry out effective therapy without necessarily knowing all the intricacies of metabolic abnormalities. There appears to be good agreement on several major points: the treatment for Addison's disease, the treatment for Cushing's syndrome, treatment for adrenogenital syndrome, the possible areas of usefulness, or exploration at least, of adrenalectomy and hypophysectomy.

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